Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

Proficiency Test Report
AQA 19-05
Chlorophyll a in Water

May 2019

ACKNOWLEDGMENTS

This study was conducted by the National Measurement Institute (NMI). Support funding was provided by the Australian Government Department of Industry, Innovation and Science.

I would like to thank the management and staff of the participating laboratories for supporting the study. It is only through widespread participation that we can provide an effective service to laboratories.

The assistance of the following NMI staff members in the planning, conduct and reporting of the study is acknowledged.

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SUMMARY

This report presents the results of the proficiency testing study AQA 19-05 – Chlorophyll a in Water. The study focused on the measurement of: chlorophyll a in water. Measurements of pheeophytin a in water were also included in this study as a measure of chlorophyll a degradation.

Two samples were prepared: Samples S1 and S2 - each consisted of a set of three filters.

Thirty five laboratories registered to participate and all but one submitted results.

The assigned value was the robust average of participants' results. The associated uncertainty was estimated from the robust standard deviation of the participants' results.

The outcomes of the study were assessed against the aims as follows:

i. assess the variability of measurements of chlorophyll a and pheophytin a in water;

Laboratory performance on the measurement of chlorophyll a was assessed using both z-scores and E_n-scores.

Of 62 z-scores, 55 (89%) were satisfactory with $|z| \leq 2$.

Of 62 E_n-scores, 46 (74%) were satisfactory with $|En| \leq 1$.

ii. Evaluate the laboratories' methods in the identification and measurement of chlorophyll a in water;

There was no significant difference between chlorophyll a results from acetone extraction and chlorophyll a results from ethanol and methanol extraction.

iii. evaluate within laboratory reproducibility;

Samples S1 and S2 were blind duplicates. Although chlorophyll a measurements are challenging, with most preparation steps to be conducted in subdued light, the results reported by participants in the two samples were in excellent agreement.

iv. Develop the practical application of traceability and measurement uncertainty and provide participants with information that will be useful in assessing their uncertainty estimates.

The assigned value is not traceable to any external reference; it is traceable to the consensus of participants' results deriving from a variety of measurement methods and (presumably) a variety of calibrators.

All but 7 numerical results were reported with an expanded measurement uncertainty. Some participants attached an estimate of the expanded measurement uncertainty to a result reported as less than their limit of reporting.

v. Develop the practical application of traceability and measurement uncertainty and produce materials that can be used in method validation and as control samples.

The study samples were checked for homogeneity and stability and are well characterised, both by in-house testing and from the results of the proficiency round. Surplus test samples are available for sale.

1 INTRODUCTION

1.1 NMI Proficiency Testing Program

The National Measurement Institute (NMI) is responsible for Australia's national measurement infrastructure, providing a range of services including a chemical proficiency testing program.

Proficiency testing (PT) is: 'evaluation of participant performance against pre-established criteria by means of interlaboratory comparison.'[1] NMI PT studies target chemical testing in areas of high public significance such as trade, environment and food safety. NMI offers studies in:

- inorganic analytes in soil, water, food and pharmaceuticals;
- pesticide residues in fruit and vegetables, soil and water;
- petroleum hydrocarbons in soil and water;
- PFAS in soil, water and biota;
- allergens in food;
- controlled drug assay; and
- folic acid in flour.

AQA 19-05 is the third NMI proficiency test of the analysis of chlorophyll a in water.

1.2 Study Aims

The aims of the study were to:

- assess the variability of measurements of chlorophyll a in water;
- evaluate the laboratories methods used in determination of chlorophyll a and pheophytin a in water;
- evaluate within-laboratory repeatability; and
- develop the practical application of traceability and measurement uncertainty and provide participants with information that will be useful in assessing their uncertainty estimates.

1.3 Study Conduct

The conduct of NMI proficiency tests is described in the NMI Chemical Proficiency Testing Study Protocol.[2] The statistical methods used are described in the NMI Chemical Proficiency Statistical Manual.[3] These documents have been prepared with reference to ISO Standard 17043[1] and The International Harmonized Protocol for Proficiency Testing of (Chemical) Analytical Laboratories.[4]

NMI is accredited by the National Association of Testing Authorities, Australia (NATA) to ISO 17043 as a provider of proficiency testing schemes. This scheme is within the scope of NMI's accreditation.

The choice of the test method was left to the participating laboratories with the following stipulations: (1) all procedures were to be carried out under subdued light to prevent photo-decomposition, and (2) use 90% (v/v) acetone as the extraction solution.

2 STUDY INFORMATION

2.1 Selection of Matrices and Analytes

The study was based on participants' expressions of interest and was intended to help laboratories to assess their methods for chlorophyll a measurements in water.

2.2 Participation

Thirty five laboratories registered to participate and all but one submitted results.

The timetable of the study was:

Invitation issued:	4 March 2019
Samples dispatched:	25 March 2019
Results due:	5 April 2019
Interim report issued:	10 April 2019

2.3 Test Material Specification

Two samples were provided for analysis:

Samples S1 and S2 consisted of three glass fibre filters each:

- 1L of water was filtered through 0.45 µm glass fibre filter. The sample taken from the water on the filter was placed on an airtight brown container, wrapped in aluminium foil and stored frozen in the dark.

As Samples S1 and S2 were blind duplicates, the chlorophyll a concentration in the two study samples was expected to be the same.

2.4 Laboratory Code

All laboratories that agreed to participate were assigned a confidential code number.

2.5 Sample Preparation, Analysis and Homogeneity Testing

Homogeneity testing was conducted for chlorophyll a in Samples S1 and S2 by Chemcentre. The preparation and analysis are described in Appendix 1. The sample was found to be sufficiently homogeneous for the assessment of participants' results.

2.6 Stability of Analytes

A stability testing was carried out for chlorophyll a in the study samples. This is described in Appendix 3. The samples were found to be sufficiently stable for the assessment of participants' results.

2.7 Sample Storage, Dispatch and Receipt

Samples S1 and S2 were stored at -20°C and dispatched by courier on 25 March 2019.

A description of the test sample, instructions to participants, and a form for participants to confirm the receipt of the test sample were sent with the sample.

An Excel spreadsheet for the electronic reporting of results was emailed to participants.

2.8 Instructions to Participants

Participants were instructed as follows:

- Participants are advised to start analyses as soon as they receive the samples; if this is not possible than the sample should be stored frozen.
- Participants are asked to record the date when the analyses were conducted.
- All procedures should be carried out under subdued light to prevent photo-decomposition.
- Quantitatively analyse the samples using your normal test method but use 90% (v/v) acetone as extraction solution.

- Participants are asked to analyse and report results for each filter (regardless the order) in units of µg/L. However the average of the three results will be used for scoring
- Report results using the electronic results sheet emailed to the participant;
- Report results for:

Tests	Approximate Concentration Range (ug/L)
S1 chlorophyll a	1-30
S1 pheophytin a	NA
S2 chlorophyll a	2.5-50
S2 pheophytin a	NA

NA-not available

- Report results as you would report to a client. This is the figure that will be used in all statistical analysis in the study report.
- For each analyte in each sample, report the expanded measurement uncertainty associated with your analytical result (e.g. 5.02 ± 0.51 µg/L)
- Please send us the requested details regarding the test method and the basis of your uncertainty estimate.
- Return the completed results sheet by email (proficiency@measurement.gov.au).

An interim report was emailed to participants on 10 April 2019

3 PARTICIPANT LABORATORY INFORMATION

3.1 Test Method Summaries

Summaries of test methods are transcribed in Table 1 and Table 2.

Table 1 Methodology

Lab. Code	Method Reference	Disruption Method	Disruption Time	Extraction Time	Extraction Agent	Vol (mL)
1	APHA 10200 H 2 (spectrophotometer)	Sonication	100 sec	overnight	90% acetone	30
2	In House	Grinding	Until macerated	Overnight (8-12 hours)	90% acetone	15
3	ISO/DIS 10260, Method for Photometric determination of Chlorophyll a, 1991	Heating with ethanol	5 min		90% ethanol	20
4	APHA 2 10200H	Sonication	15 min	Overnight (8-12 hours	90% acetone	9
5	In house 46 (based on APHA 10200H)	Waterbath at 75 °C (shaken)	5 min		90% ethanol	10
6*	SCOR - UNESCO	Sonication	25 min	3 to 24 hours	90% acetone	10
7*	ISO 1026:1992 (E)	Other	5 min		90%acetone	20
8	APHA 10200H	Grinding	Until well ground		90% acetone	10
9*	APHA 23rd edition 10200H	Grinding	1 min		Acetone solution	9
10	APHA 10200H	Grinding	5 min	1-2 hours	90% acetone	10

Table 1 Methodology (continued)

Lab. Code	Method Reference	Disruption Method	Disruption Time	Extraction Time	Extraction Agent	Vol (mL)
11	APHA 10200H	Grinding	2 min		90% acetone	10
12	APHA 10200 H Chlorophyll	Grinding	5 min	2 hours	90% Acetone	8
13	APHA	grinding			90% acetone	10
14	APHA	grinding	2 hours		Aqueous acetone	10
15	Standard Methods for the Examination of Water and Wastewater, APHA. Method 10200 H.	Shaking	1 min	2 hours	90%acetone	20
16	APHA-10200H	Grinding	Until complete		90% acetone	10
17	APHA-20200H	Heat	3 min		90% methanol	16
18*	8.1. Limnology and oceanography (1967) No. 12 p343-346	Sonication	15 min		90% acetone	10
19	ISO 10260 (1992) for chlorophyll a and pheophytin	Vortex @ 1800 RPM	1 min		96% ethanol	10
20	APHA 10200H	Grinding	1 min		90% acetone	10
22	APHA 21st edition 10200H (2005)	Grinding			90% acetone	10
23	In house based on APHA 10200H	Grinding	20 sec	Overnight	90% acetone	10
24	ISO 10260 (1992) for chlorophyll a and pheophytin	Vortex @ 1800 RPM	1 min		96% ethanol	10
25	APHA 10200H and USEPA 446.0	Sonication	20 min	16 hours	90% acetone	10
26	APHA 10200H	Sonication	1 min	overnight	90% acetone	8
27	APHA 10200H 23rd edition	Grinding	1 min		90% acetone	10
28	APHA 10200H	Grinding	1 min	2 hours	90% acetone	10
29	APHA 10200H chlorophyll	Tissue homogeniser	Until complete		90% acetone	50
30*	ISO 10260 (1992) Rev 2017	Nil	Nil	overnight	90% acetone	15
31	APHA 10200H	Grinding	2 min		90% acetone	10
32*	APHA 10200 H 3 (spectrophotometer)	Sonication	100 sec	overnight	90% acetone	30
33	APHA 10200 H 3	Sonication	100 sec	overnight	90% acetone	30
34	EPA 445.0	Sonication	100 sec	overnight	90% acetone	30
35	APHA 10200H 23rd edition	Grinding	1 min	S2 20 hours S1 48 hours	90% acetone	10

*Additional information in Table 2

3.2 Additional Method Information

Participants had the option to report additional information for each sample analysed. These are transcribed in Table 2.

Table 2 Additional Method information

Lab. Code	Additional Information
6	Based on Trichromatic Equations
7	Absorbance was measured at 665 and 750 nm
9	Pheophytin calculated from chlorophyll a
18	Wavelengths used were 665 and 750 nm
19	The laboratory used 96% ethanol as the solvent for this proficiency round (it is the solvent used for the routine method).
23	Extraction done at 1-4 °C
24	The laboratory used 96% ethanol as the solvent for this proficiency round (it is the solvent used for the routine method).
27	Samples frozen at -80 °C
30	Magnesium carbonate was not used
32	Trichromatic method
34	"Uncorrected" chl a section 12.1 in EPA 445.0

3.3 Instruments Used for Measurements

The instruments measurement methods reported by participants are presented in Appendix 6.

3.4 Basis of Participants' Measurement Uncertainty Estimates

Participants were requested to provide information about the basis of their uncertainty estimates. Those returned are transcribed in Table 3.

Table 3 Basis of Uncertainty Estimate

Lab. Code	Approach to Estimating MU	Information Sources for MU Estimation		Guide Document for Estimating MU
		Precision[a]	Method Bias[a]	
1*	Standard deviation of replicate analyses multiplied by 2 or 3	Duplicate analysis Instrument calibration	Instrument calibration	Other
2	Professional judgement			
3	Top Down – reproducibility (standard deviation) from PT studies used directly	Duplicate analysis Instrument calibration		Eurachem/CITAC Guide
4	Top Down - precision and estimates of the method and laboratory bias	Duplicate analysis		NATA Technical Note 33
5	Standard deviation of replicate analyses multiplied by 2 or 3	Duplicate analysis Instrument calibration	Instrument calibration	ISO/GUM
6	Top Down - precision and estimates of the method and laboratory bias	Duplicate analysis	Instrument calibration	NMI uncertainty course
7	There was not enough data points to estimate measurement of uncertainty for the samples			
8	Top Down - precision and estimates of the method and laboratory bias	Control Samples - CRM Duplicate analysis	CRM	Eurachem/CITAC Guide

Table 3 Basis of Uncertainty Estimate (continued)

Lab. Code	Approach to Estimating MU	Information Sources for MU Estimation		Guide Document for Estimating MU
		Precision[a]	Method Bias[a]	
9	Top Down - precision and estimates of the method and laboratory bias	Duplicate analysis		IANZ technical guide
10	Top Down - precision and estimates of the method and laboratory bias	Control Samples – SS	Recoveries of SS	NATA Technical Note 33
11	Bottom Up (ISO/GUM, fish bone / cause and effect diagram)			Eurachem/CITAC Guide
12	Bottom Up (ISO/GUM, fish bone / cause and effect diagram)	Control Samples – SS Duplicate analysis		NMI uncertainty course
13	Top Down – reproducibility (standard deviation) from PT studies used directly	Duplicate analysis Instrument calibration		Eurachem/CITAC Guide
14	Top Down - precision and estimates of the method and laboratory bias	Control Samples - RM Duplicate analysis Instrument calibration	CRM Instrument calibration Laboratory bias from PT studies	NATA Technical Note 33
15	Standard deviation of replicate analyses multiplied by 2 or 3	Duplicate analysis	Laboratory bias from PT studies	NATA Technical Note 33
16	Bottom Up (ISO/GUM, fish bone / cause and effect diagram)	Control Samples - CRM Duplicate analysis	CRM	NATA Technical Note 33
17	Other	Control Sample Duplicate analysis Instrument calibration	Instrument calibration	Other
18	Top Down - precision and estimates of the method and laboratory bias	Duplicate analysis Instrument calibration	Instrument calibration	Eurachem/CITAC Guide
19	Top Down – reproducibility (standard deviation) from PT studies used directly	Duplicate analysis Instrument calibration	Laboratory bias from PT studies Instrument calibration	Eurachem/CITAC Guide
20	Top Down - precision and estimates of the method and laboratory bias	Instrument calibration	CRM Instrument calibration	NATA Technical Note 33
22	Top Down - precision and estimates of the method and laboratory bias	Duplicate analysis	Instrument calibration	Eurachem/CITAC Guide
23	Top Down - precision and estimates of the method and laboratory bias	Control Samples - RM		Armishaw 2002-3
24	Top Down – reproducibility (standard deviation) from PT studies used directly	Duplicate analysis Instrument calibration	Instrument calibration	Eurachem/CITAC Guide
25*	Standard deviation of replicate analyses multiplied by 2 or 3	Duplicate analysis		NATA Technical Note 33
26	Top Down - precision and estimates of the method and laboratory bias	Duplicate analysis		NATA Technical Note 33
27	Standard deviation of replicate analyses multiplied by 2 or 3	RM Duplicate analysis	Matrix Effects	NATA Technical Note 33
28	Professional judgement			
29	Bottom Up (ISO/GUM, fish bone / cause and effect diagram)	Duplicate analysis Instrument calibration	Instrument calibration	ISO/GUM
30	Top Down - precision and estimates of the method and laboratory bias	Instrument calibration	Laboratory bias from PT studies Instrument calibration	NATA Technical Note 33

Table 3 Basis of Uncertainty Estimate (continued)

Lab. Code	Approach to Estimating MU	Information Sources for MU Estimation		Guide Document for Estimating MU
		Precision[a]	Method Bias[a]	
31	Top Down - precision and estimates of the method and laboratory bias	Control Sample - RM Duplicate analysis Instrument calibration		Eurachem/CITAC Guide
32*	Standard deviation of replicate analyses multiplied by 2 or 3	Duplicate analysis Instrument calibration	Instrument calibration	Other
33*	Professional judgement			Other
34*	Professional judgement	Instrument calibration	Instrument calibration	Other
35	Standard deviation of replicate analyses multiplied by 2 or 3	RM Duplicate analysis	Matrix Effects	NATA Technical Note 33

[a] RM = Reference Material, CRM = Certified Reference Material, SS =Spiked Samples. *Additional information in Table 4.

3.5 Additional Uncertainty Information

Participants had the option to report additional information for each sample analysed. These are transcribed in Table 4.

Table 4 Additional Uncertainty information

Lab. Code	Additional Information
1	The guide document used was US EPA's Electronic code of federal regulations: 40 CFR, part 136, appendix B 'Definition and procedure for the determination of the method detection limit, revision 1.11'
25	A duplicate pairs approach was taken, with 7 duplicate pairs analysed from 3 different water matrix types (saline, river and sewage), compared with the average of the individual reads
32	The guide document used was US EPA's Electronic code of federal regulations: 40 CFR, part 136, appendix B 'Definition and procedure for the determination of the method detection limit, revision 1.11'
33	The guide document used was US EPA's Electronic code of federal regulations: 40 CFR, part 136, appendix B 'Definition and procedure for the determination of the method detection limit, revision 1.11'
34	The guide document used was US EPA's Electronic code of federal regulations: 40 CFR, part 136, appendix B 'Definition and procedure for the determination of the method detection limit, revision 1.11'

3.6 Participant Comments on this PT Study or Suggestions for Future Studies

The study coordinator welcomes comments or suggestions from participants about this study or possible future studies. Such feedback may be useful in improving future studies. Participants' comments are reproduced in Table 5.

Table 5 Participants' Comments

Participants' Comments	Study Co-ordinator's Response
After extraction, S1 matrix for all three filters were slightly greener than S2. Extracted in 90% Methanol as per our routine method of analysis. Excitation results of S1 is also bit higher than S2 in Fluorometer analyses.	The robust average of participants' results in the two samples were not significantly different and were in good agreement with spike value and homogeneity value (see Appendix 1).

Table 5 Participants' Comments (continued)

Participants' Comments	Study Co-ordinator's Response
Given the distance that this sample travelled to NZ we have no means to assess if temperature conditions exceeded what was required. We only know this sample arrived and was promptly placed in the quarantine section of our freezer as is usual for international samples. Suggest that perhaps a temperature data logger be packed along with the sample in the future.	The sample was packed in 3 layers of ice packs; The inner ice pack in contact with the sample jar was reported as frozen in your laboratory feedback form. A temperature data logger would indicate the temperature in the box and not in the jar. The study conduct was validated through a series of previous trials, through previous PT studies and in the present study through stability testing (see Appendix 3).
Samples were analysed in subdued light, but filter papers and extracts were very pale, not sure if the samples degraded during transit.	

4 PRESENTATION OF RESULTS AND STATISTICAL ANALYSIS

4.1 Results Summary

Participant results are listed in Tables 6 to 9 with resultant summary statistics: robust average, median, maximum, minimum, robust standard deviation (SD_{rob}) and robust coefficient of variation (CV_{rob}).

Bar charts of results and performance scores are presented in Figures 2 to 5.

An example chart with interpretation guide is shown in Figure 1.

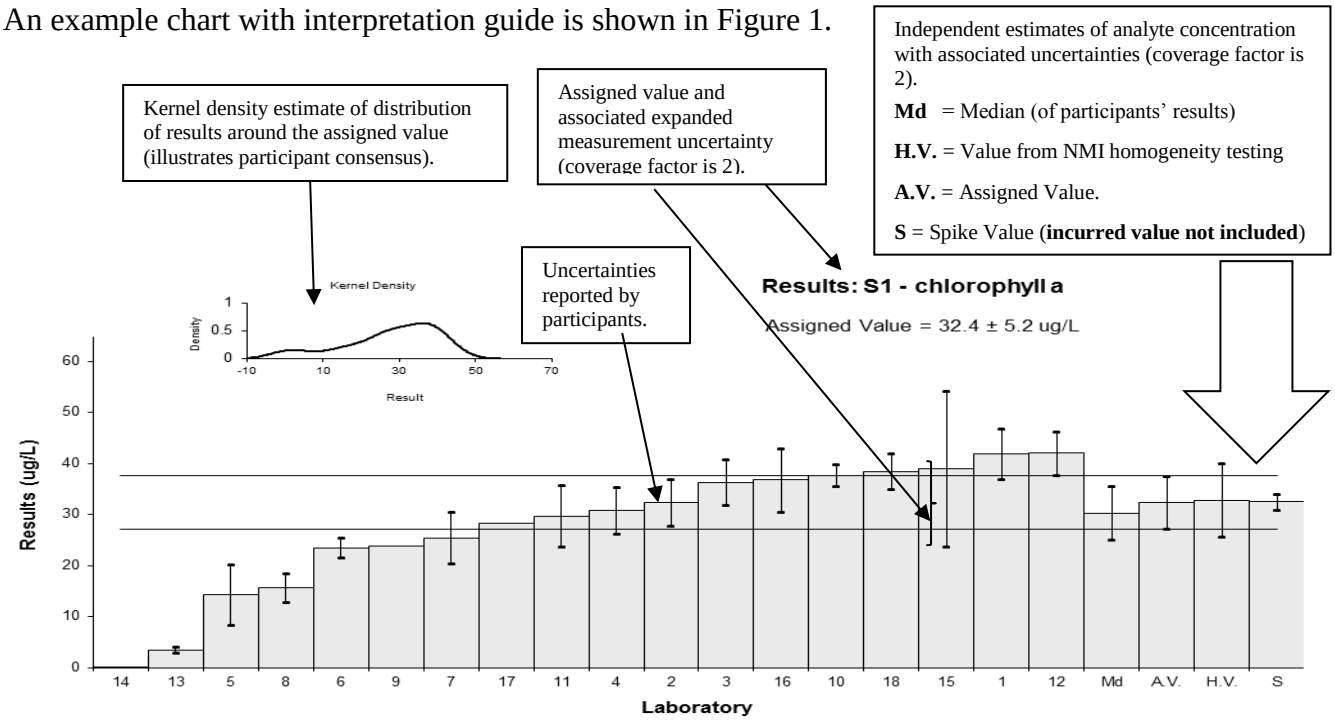


Figure 1 Guide to Presentation of Results

4.2 Assigned Value

An example of an assigned value calculation using data from the present study is given in Appendix 3. The assigned value is defined as: 'the value attributed to a particular property of a proficiency test item.'[1] In this study, the property is the mass fraction of analyte. Assigned values were the robust average of participants' results; the expanded uncertainties were estimated from the associated robust standard deviations.

4.3 Robust Average

The robust averages and associated expanded measurement uncertainties were calculated using the procedure described in 'Statistical methods for use in proficiency testing by interlaboratory comparisons, ISO13528:2015(E)'.[5]

4.4 Robust Between-Laboratory Coefficient of Variation

The robust between-laboratory coefficient of variation (robust CV) is a measure of the variability of participants' results and was calculated using the procedure described in ISO 13528:2015(E).[5]

4.5 Target Standard Deviation

The target standard deviation (σ) is the product of the assigned value *(X)* and the performance coefficient of variation (PCV) as presented in Equation 1.

$$\sigma = (X) * PCV \qquad \text{Equation 1.}$$

This value is used for calculation of participant z-score and provides scaling for laboratory deviation from the assigned value. It is important to note that the PCV is a fixed value and is not the standard deviation of participants' results. The fixed value set for PCV is based on the existing regulation, the acceptance criteria indicated by the methods, the matrix, the concentration level of analyte and on experience from previous studies. It is backed up by mathematical models such as Thompson Horwitz equation.[6] By setting a fixed and realistic value for PCV, the participant's performance does not depend on other participants' performance and can be compared from study to study and against achievable performance.

4.6 z-Score

An example of z-score calculation using data from the present study is given in Appendix 2.

For each participant's result a z-score is calculated according to Equation 2 below:

$$z = \frac{(\chi - X)}{\sigma} \qquad \text{Equation 2}$$

where:

- z is z-score
- χ is participant's result
- X is the assigned value
- σ is the target standard deviation

A z-score with absolute value (|z|):

- $|z| \leq 2$ is satisfactory;
- $2 < |z| < 3$ is questionable;
- $|z| \geq 3$ is unsatisfactory.

4.7 E_n-Score

An example of E_n-score calculation using data from the present study is given in Appendix 2.

The E_n-score is complementary to the z-score in assessment of laboratory performance. E_n-score includes measurement uncertainty and is calculated according to Equation 3 below:

$$E_n = \frac{(\chi - X)}{\sqrt{U_\chi^2 + U_X^2}} \qquad \text{Equation 3}$$

where:

- E_n is E_n-score
- χ is a participant's result
- X is the assigned value
- U_χ is the expanded uncertainty of the participant's result
- U_X is the expanded uncertainty of the assigned value

An E_n-score with absolute value ($|E_n|$):

- $|E_n| \leq 1$ is satisfactory;
- $|E_n| > 1$ is unsatisfactory.

4.8 Traceability and Measurement Uncertainty

Laboratories accredited to ISO/IEC Standard 17025:2017[7] must establish and demonstrate the traceability and measurement uncertainty associated with their test results. Guidelines for quantifying uncertainty in analytical measurement are described in the Eurachem/CITAC Guide.[8]

5 TABLES AND FIGURES

Table 6

Sample Details

Sample No.	S1
Matrix.	Water
Analyte.	Chlorophyll a
Units	ug/L

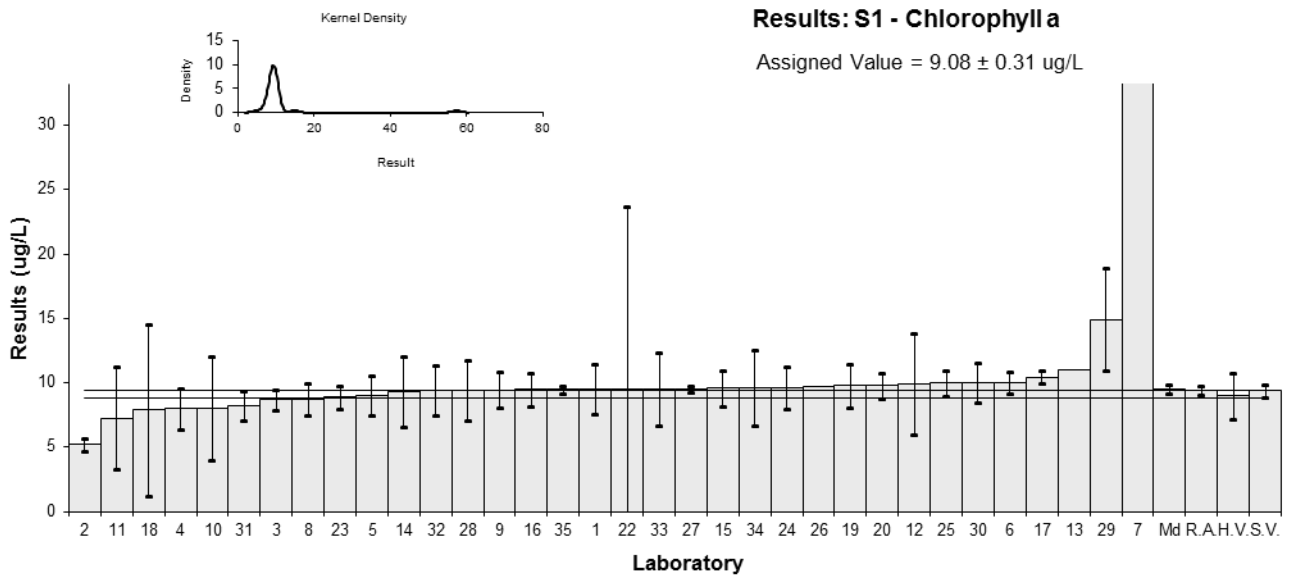
Participant Results

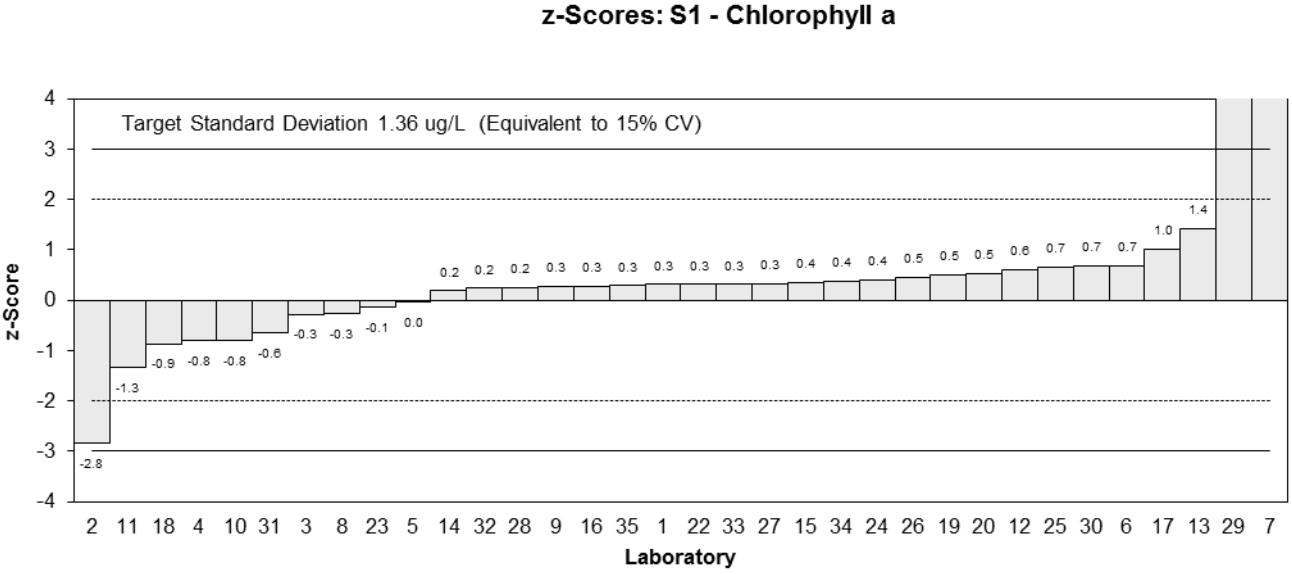
Lab Code	Result	Uncertainty	z-Score	E_n-Score
1	9.5	1.9	0.31	0.22
2	5.2	0.5	-2.85	-6.6
3	8.7	0.8	-0.28	-0.44
4	7.98	1.56	-0.81	-0.69
5	9.02	1.55	-0.04	-0.04
6	10.01	0.85	0.68	1.03
7	57.4	NR	35.48	155.87
8	8.72	1.23	-0.26	-0.28
9	9.45	1.42	0.27	0.25
10	8	4	-0.79	-0.27
11	7.25	4	-1.34	-0.46
12	9.9	3.9	0.6	0.21
13	11	NR	1.41	6.19
14	9.33	2.748	0.18	0.09
15	9.56	1.4	0.35	0.33
16	9.463	1.29	0.28	0.29
17	10.45	0.53	1.01	2.23
18	7.9	6.67	-0.87	-0.18
19	9.76	1.67	0.5	0.4
20	9.79	1	0.52	0.68
22	9.5	14.2	0.31	0.03
23	8.9	0.9	-0.13	-0.19
24	9.62	1.65	0.4	0.32
25	9.97	1.00	0.65	0.85
26	9.7	NR	0.46	2
27	9.53	0.264	0.33	1.11
28	9.42	2.36	0.25	0.14
29	14.91	4	4.28	1.45
30	10	1.50	0.68	0.6
31	8.2	1.12	-0.65	-0.76
32	9.4	1.9	0.23	0.17
33	9.5	2.8	0.31	0.15
34	9.6	2.9	0.38	0.18
35	9.47	0.264	0.29	0.96

Statistics

Assigned Value*	9.08	0.31
Spike	9.38	0.47
Homogeneity Value	9.0	1.8
Robust Average	9.38	0.37
Median	9.50	0.34
Mean	10.8	
N	34	
Max.	57.4	
Min.	5.2	
Robust SD	0.92	
Robust CV	9.8%	

*The assigned value was calculated as the robust average of the combined results of duplicate Samples S1 and S2 excluding Laboratories, 7 and 29.


Kernel Density
Density
Result
Results: S1 - Chlorophyll a
Assigned Value = 9.08 ± 0.31 ug/L
Results (ug/L)
Laboratory
2 11 18 4 10 31 3 8 23 5 14 32 28 9 16 35 1 22 33 27 15 34 24 26 19 20 12 25 30 6 17 13 29 7 Md R.A.H.V.S.V.


z-Scores: S1 - Chlorophyll a
Target Standard Deviation 1.36 ug/L (Equivalent to 15% CV)
z-Score
Laboratory
2 11 18 4 10 31 3 8 23 5 14 32 28 9 16 35 1 22 33 27 15 34 24 26 19 20 12 25 30 6 17 13 29 7

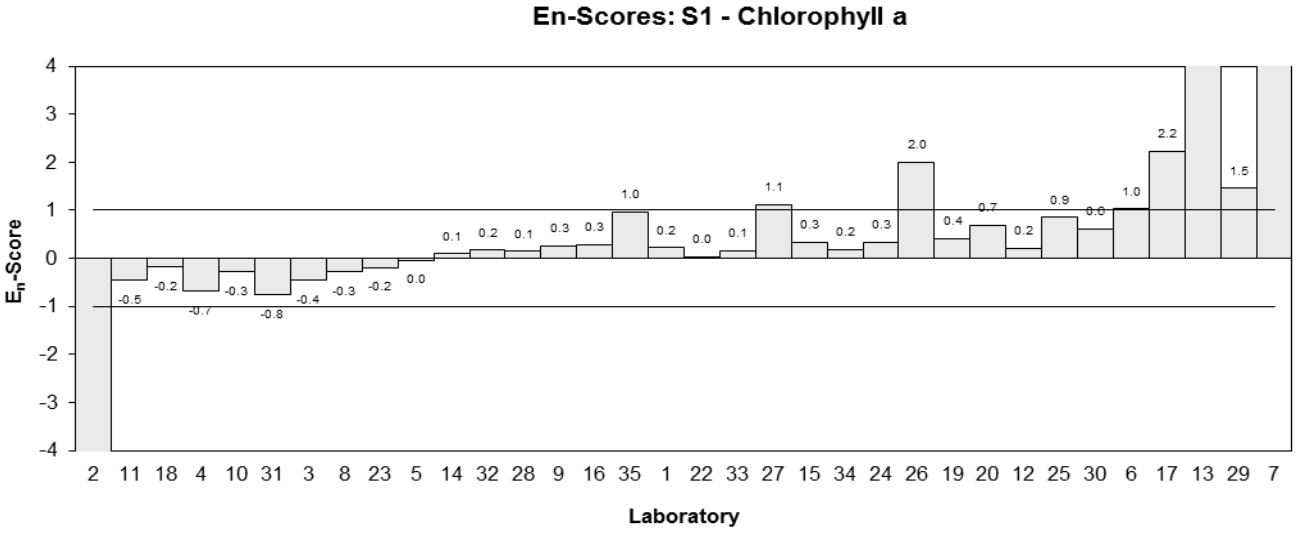

En-Scores: S1 - Chlorophyll a
En-Score
Laboratory
2 11 18 4 10 31 3 8 23 5 14 32 28 9 16 35 1 22 33 27 15 34 24 26 19 20 12 25 30 6 17 13 29 7

Figure 2

Table 7

Sample Details

Sample No.	S1
Matrix.	Water
Analyte.	Pheophytin a
Units	ug/L

Participant Results

Lab Code	Result	Uncertainty
1	<4	NR
2	12.7	1
3	1.7	0.3
4	1.12	0.56
5	NT	NT
6	<0.2	NR
7	NR	NR
8	<1	0.14
9	0.64	0.10
10	2	2
11	2.5	2
12	<1	NR
13	NR	NR
14	<1	NR
15	NT	NT
16	<1	0.5
17	NT	NT
18	NT	NT
19	9.32	3.19
20	<5	1
22	0.0	NR
23	0.9	0.5
24	1.33	0.45
25	NT	NT
26	NR	NR
27	<2.0	0.255
28	<0.5	0.5
29	<0.5	4
30	<2	NR
31	NT	NT
32	NT	NT
33	<4	NR
34	NT	NT
35	<2.0	0.255

Statistics

Assigned Value	Not Set	
Spike	Not Spiked	
Robust Average	1.9	1.4
Median	1.5	0.8
Mean	3.2	
N	10	
Max.	12.7	
Min.	0	
Robust SD	1.8	
Robust CV	93%	

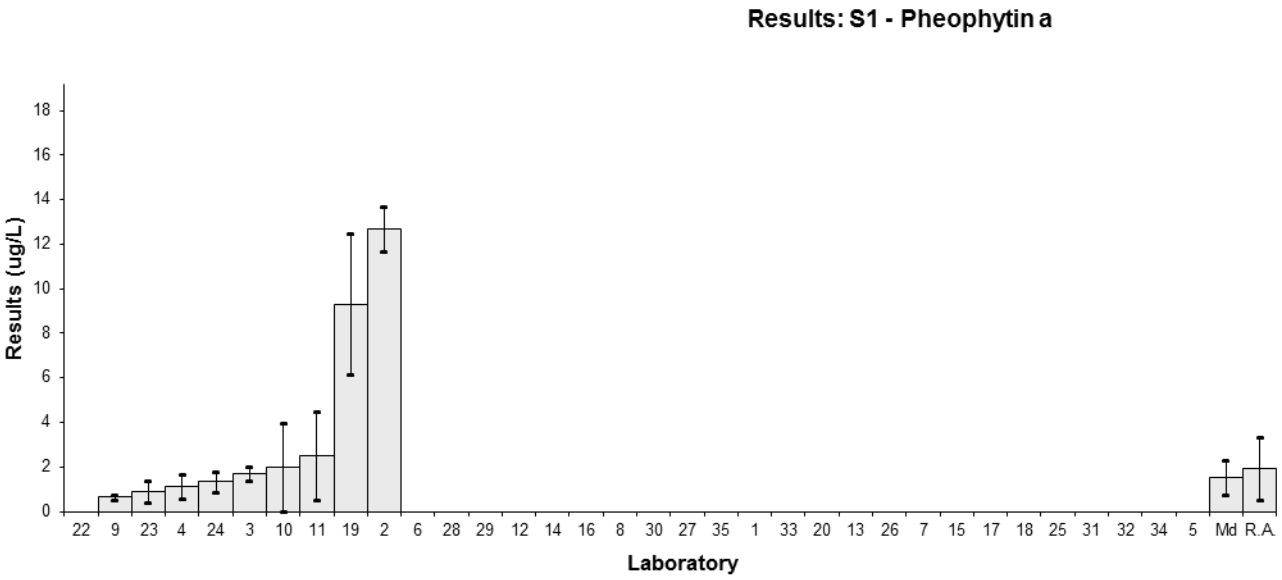

Results: S1 - Pheophytin a
Results (ug/L)
0
2
4
6
8
10
12
14
16
18
22 9 23 4 24 3 10 11 19 2 6 28 29 12 14 16 8 30 27 35 1 33 20 13 26 7 15 17 18 25 31 32 34 5 Md R.A.
Laboratory

Figure 3

Table 8

Sample Details

Sample No.	S2
Matrix.	Water
Analyte.	Chlorophyll a
Units	ug/L

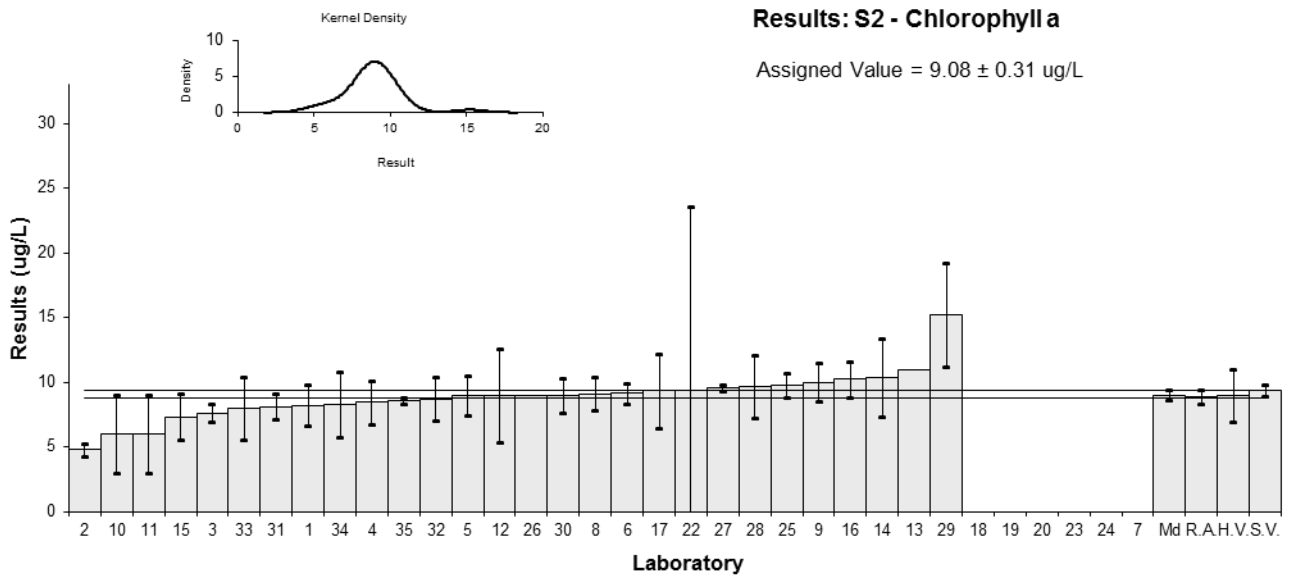
Participant Results

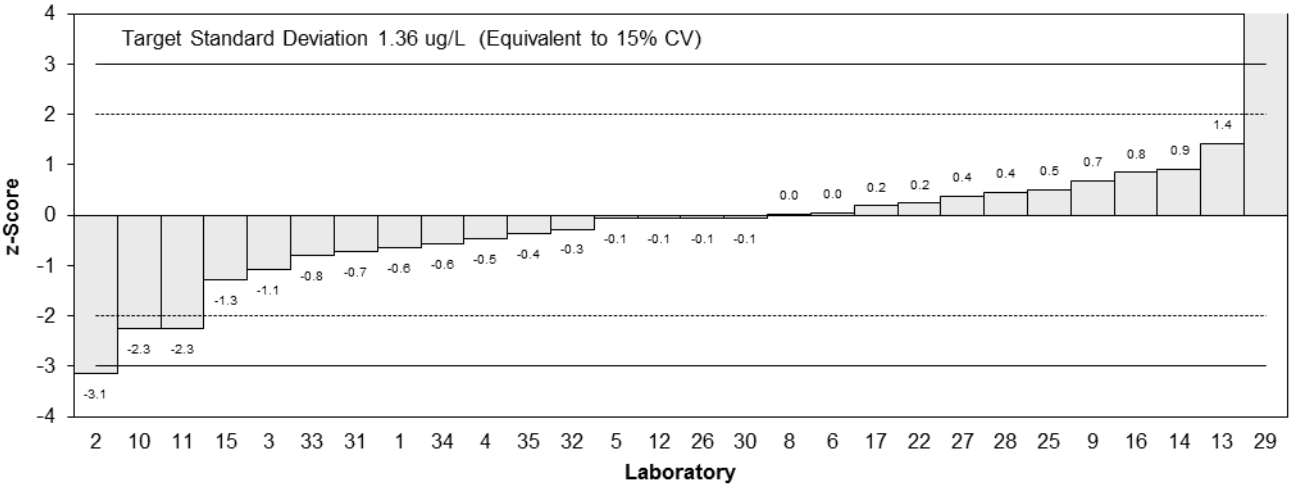
Lab Code	Result	Uncertainty	z-Score	En-Score
1	8.2	1.6	-0.65	-0.54
2	4.8	0.5	-3.14	-7.28
3	7.6	0.7	-1.09	-1.93
4	8.43	1.65	-0.48	-0.39
5	8.99	1.55	-0.07	-0.06
6	9.14	0.77	0.04	0.07
7	NT	NT		
8	9.09	1.28	0.01	0.01
9	10.0	1.5	0.68	0.6
10	6	3	-2.26	-1.02
11	6	3	-2.26	-1.02
12	9.0	3.6	-0.06	-0.02
13	11	NR	1.41	6.19
14	10.33	3.033	0.92	0.41
15	7.32	1.8	-1.29	-0.96
16	10.23	1.38	0.84	0.81
17	9.33	2.91	0.18	0.09
18	NT	NT		
19	NT	NT		
20	NT	NT		
22	9.4	14.2	0.23	0.02
23	NT	NT		
24	NT	NT		
25	9.77	0.98	0.51	0.67
26	9	NR	-0.06	-0.26
27	9.59	0.264	0.37	1.25
28	9.69	2.42	0.45	0.25
29	15.24	4	4.52	1.54
30	9	1.35	-0.06	-0.06
31	8.1	1.01	-0.72	-0.93
32	8.7	1.7	-0.28	-0.22
33	8.0	2.4	-0.79	-0.45
34	8.3	2.5	-0.57	-0.31
35	8.59	0.264	-0.36	-1.2

Statistics

Assigned Value*	9.08	0.31
Spike	9.38	0.47
Homogeneity Value	9.0	1.8
Robust Average	8.87	0.57
Median	9.00	0.42
Mean	8.89	
N	28	
Max.	15.24	
Min.	4.8	
Robust SD	0.92	
Robust CV	10%	

*The assigned value was calculated as the robust average of the combined results of duplicate Samples S1 and S2. Excluding Laboratories 7 and 29.


Kernel Density
Density
Result
Results: S2 - Chlorophyll a
Assigned Value = 9.08 ± 0.31 ug/L
Results (ug/L)
2 10 11 15 3 33 31 1 34 4 35 32 5 12 26 30 8 6 17 22 27 28 25 9 16 14 13 29 18 19 20 23 24 7 Md R.A.H.V.S.V.
Laboratory


z-Scores: S2 - Chlorophyll a
Target Standard Deviation 1.36 ug/L (Equivalent to 15% CV)
z-Score
2 10 11 15 3 33 31 1 34 4 35 32 5 12 26 30 8 6 17 22 27 28 25 9 16 14 13 29
Laboratory

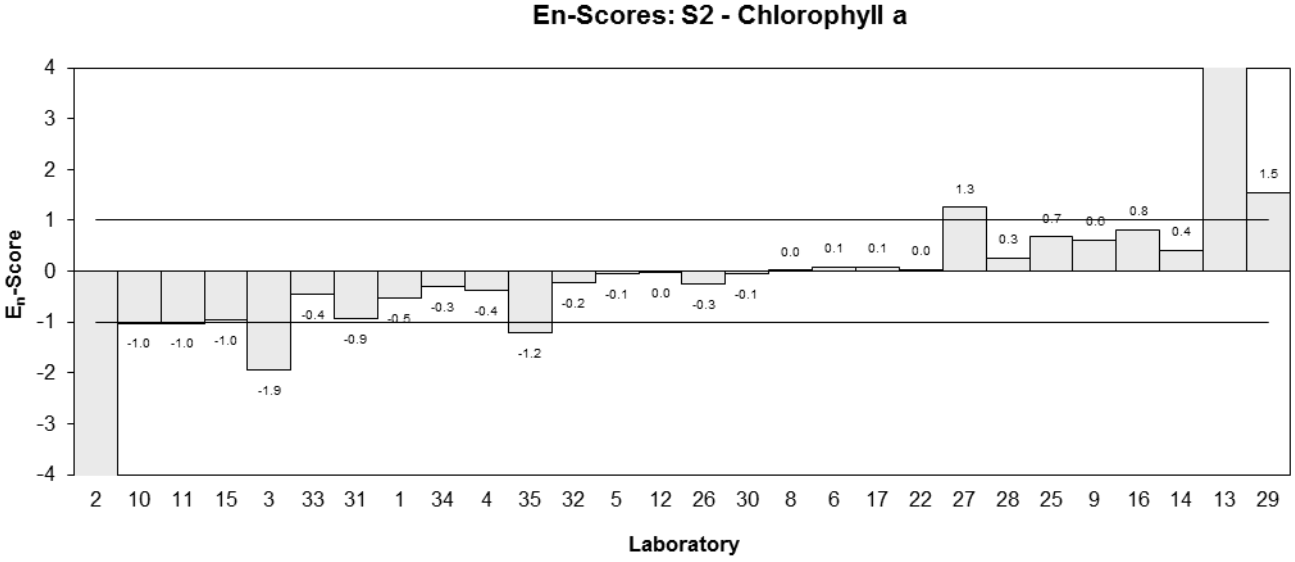

En-Scores: S2 - Chlorophyll a
En-Score
2 10 11 15 3 33 31 1 34 4 35 32 5 12 26 30 8 6 17 22 27 28 25 9 16 14 13 29
Laboratory

Figure 4

Table 9

Sample Details

Sample No.	S2
Matrix.	Water
Analyte.	Pheophytin a
Units	ug/L

Participant Results

Lab Code	Result	Uncertainty
1	<4	NR
2	13.8	1
3	2.3	0.4
4	0.79	0.4
5	NT	NT
6	<0.2	NR
7	NT	NT
8	<1	0.14
9	0.16	0.02
10	3	3
11	3	2
12	<1	NR
13	NR	NR
14	<1	NR
15	NT	NT
16	<1	0.5
17	NT	NT
18	NT	NT
19	NT	NT
20	NT	NT
22	0.0	NR
23	NT	NT
24	NT	NT
25	NT	NT
26	NR	NR
27	<2.0	0.255
28	<0.5	0.5
29	<0.5	4
30	2	0.30
31	NT	NT
32	NT	NT
33	<4	NR
34	NT	NT
35	<2.0	0.255

Statistics

Assigned Value	Not Set	
Spike	Not Spiked	
Homogeneity Value	9.0	1.8
Robust Average	2.0	1.6
Median	2.2	1.4
Mean	3.1	
N	8	
Max.	13.8	
Min.	0	
Robust SD	1.9	
Robust CV	95%	

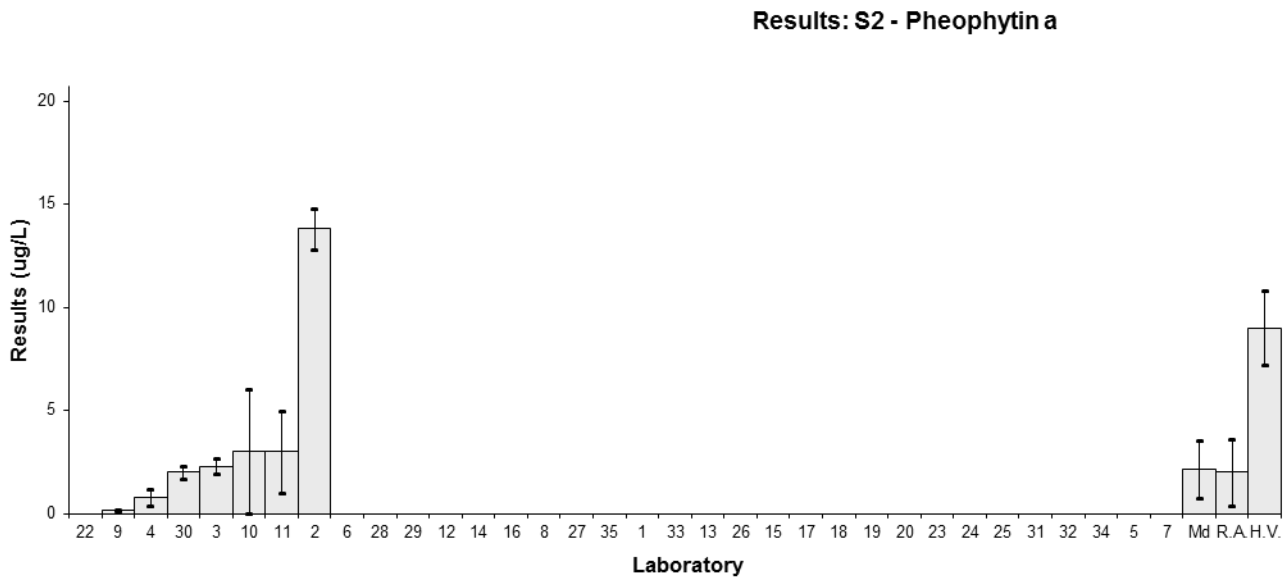

Results: S2 - Pheophytin a
Results (ug/L)
20
15
10
5
0
22 9 4 30 3 10 11 2 6 28 29 12 14 16 8 27 35 1 33 13 26 15 17 18 19 20 23 24 25 31 32 34 5 7 Md R.A. H.V.
Laboratory

Figure 5

6 DISCUSSION OF RESULTS

6.1 Assigned Value

Assigned Value for chlorophyll a in the duplicate study samples was the robust average of combined results reported for S1 and S2. The assigned value was in good agreement with the spike and homogeneity values. The robust average was used as the assigned value and its associated expanded uncertainty was calculated using the procedure described in ISO13528:2015. Results less than 50% and more than 150% of the robust average were removed before calculation of the assigned value.[5] Appendix 2 sets out the calculation for the assigned value of chlorophyll a in Samples S1 and S2 its associated uncertainty.

No assigned value was set for pheophytin a in water. This analyte was introduced only as a measure of chlorophyll a degradation.

Traceability The assigned value is not traceable to any external reference; it is traceable to the consensus of participants' results deriving from a variety of measurement methods and (presumably) a variety of calibrators. So although expressed in SI units, the metrological traceability of the assigned values has not been established.

6.2 Measurement Uncertainty Reported by Participants

Participants were asked to report an estimate of the expanded measurement uncertainty associated with their results. All but 7 numerical results were reported with an expanded measurement uncertainty, indicating that most laboratories have addressed this requirement of ISO 17025.[7] The participants used a wide variety of procedures to estimate the expanded measurement uncertainty. These are presented in Table 3.

Approaches to estimating measurement uncertainty include: standard deviation of replicate analysis, Horwitz formula, professional judgement, bottom up approach, top down approach using precision and estimates of method and laboratory bias, and top down approach using only the reproducibility from inter-laboratory comparisons studies.[9 – 14]

Proficiency tests allow a check of the reasonableness of uncertainty estimates. Results and the expanded measurement uncertainties are presented in the bar charts for each analyte (Figure 2 to 5). In this study, in some cases, the reported expanded measurement uncertainty has been over-estimated (e.g. Laboratories 18 and 22 for chlorophyll a in S1) or under-estimated (e.g. Laboratories 27 and 35 for chlorophyll a in S1). As a simple rule of thumb, when the uncertainty estimate is smaller than the assigned uncertainty value or larger than the uncertainty of the assigned value plus twice the target standard deviation then this should be viewed as suspect.

Some laboratories attached estimates of the expanded measurement uncertainty to results reported as less than their limit of detection (e.g Lab 8 for pheophtyn a in S1). An estimate of uncertainty expressed as a value cannot be attached to a result expressed as a range.

In some cases the results were reported with an inappropriate number of significant figures. The recommended format is to write uncertainty to no more than two significant figures and then to write the result with the corresponding number of decimal places. For example, instead of 8.43 ± 1.65 µg/L, it is better to report 8.4 ± 1.7 µg/L or instead of 10.33 ± 3.033 µg/L, it is better to report 10.3 ± 3.0 µg/L.[8]

6.3 z-Score

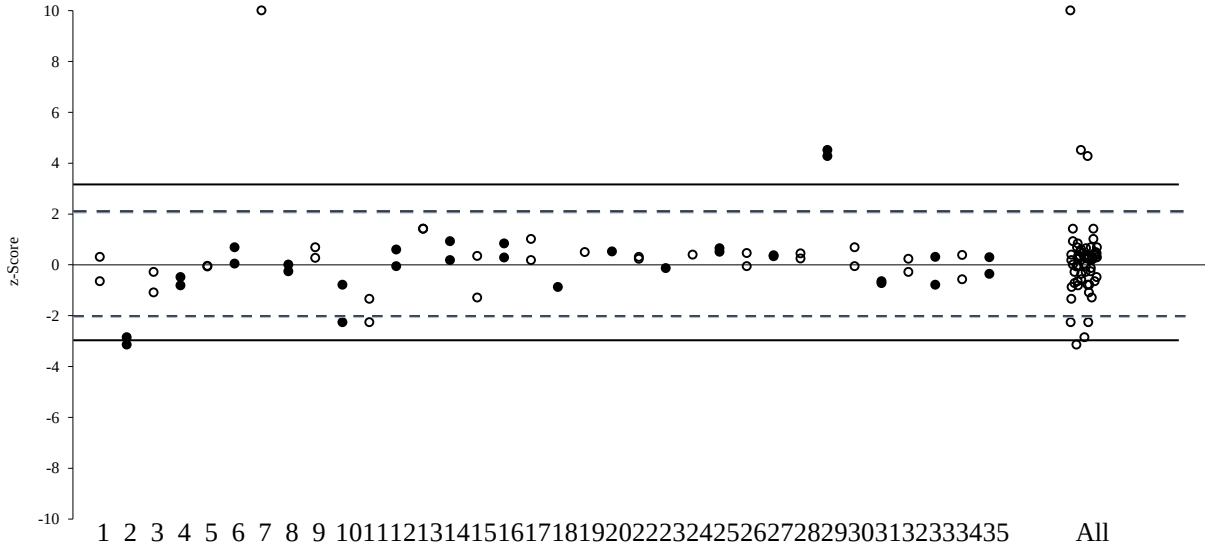
The z-score compares the participant's deviation from the assigned value with the target standard deviation set for proficiency assessment.

The target standard deviation defines satisfactory performance in a proficiency test. Target standard deviations equivalent to 15% PCV were used to calculate z-scores. Unlike the standard deviation based on between laboratories CV, setting the target standard deviation as a realistic, set value enables z-scores to be used as fixed reference value points for assessment of laboratory performance, independent of group performance. The between laboratory coefficient of variation predicted by the Thompson equation[6] and the participants' coefficient of variation resulted in this study are presented for comparison in Table 10.

Table 10 Between Laboratory CV of this study, Thompson CV and Set Target CV

Sample	Analyte	Assigned value (µg/L)	Between Laboratories CV	Thompson CV	Target SD (as CV)
S1	chlorophyll a	9.08	9.8%	22%	15%
S2	chlorophyll a	9.08	10%	22%	15%

The dispersal of participants' z-scores is presented in Figure 6. Of 62 results for which z-scores were calculated, 55 (89%) returned a satisfactory score of $|z| \leq 2$ and 3 (5%) were questionable of $2 < |z| \leq 3$.



Scores of >10 or <-10 have been plotted as 10 or -10.

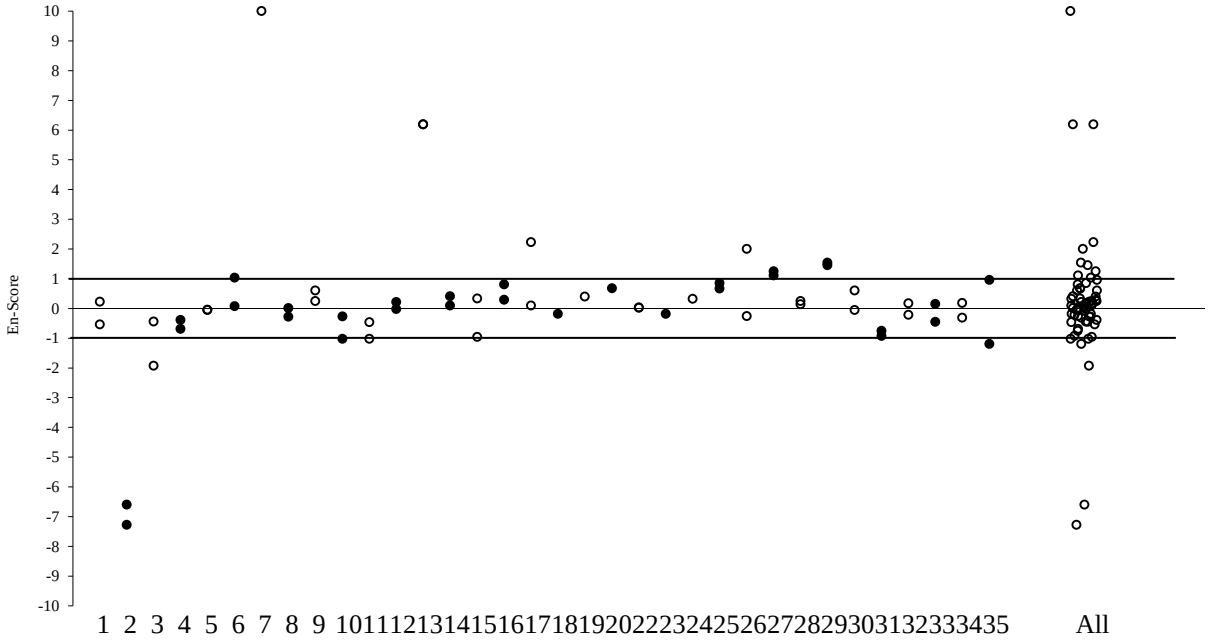
Figure 6 z-Score Dispersal by Laboratory

Participants with both z-scores larger than 2 or smaller than -2 should check for laboratory bias.

6.4 E_n-Score

E_n-score should be interpreted only in conjunction with z-scores. The E_n-score indicates how closely a result agrees with the assigned value taking into account the respective uncertainties. An unsatisfactory E_n score for an analyte can either be caused by an inappropriate measurement, an inappropriate estimation of measurement uncertainty, or both.

The dispersal of participants' E_n-scores is graphically presented in Figure 7. Where a laboratory did not report an expanded uncertainty with a result, an expanded uncertainty of zero (0) was used to calculate the E_n-score. Of 62 results for which E_n-scores were calculated, 46 (74%) returned a satisfactory score of $|E_n| \leq 1$ indicating agreement of the participants' results with the assigned values within their respective expanded measurement uncertainties.



Scores of >10 or <-10 have been plotted as 10 or -10.

Figure 7 E_n-Score Dispersal by Laboratory

6.5 Participants' Results and Analytical Methods

Samples S1 and S2 consisted of three filter papers, each fortified with the same amount of chlorophyll a standard solution. Participants were assessed on the average of three measurements. Laboratories were advised:
"… to analyse and report results for each filter (regardless the order) in units of µg/L. However the average of the three results will be used for scoring."

The two samples were blind duplicates; chlorophyll a concentration in the two samples was identical. A summary of participants' results and performance in the two study samples is presented in Table 11. Participants' results reported for each filter are presented in Tables 17 to 20.

The methods used by participants for chlorophyll a analysis in the present study are presented in Tables 1 and 2 while the measurement techniques are presented in Appendix 6.

Laboratory 14 measured the three filters at the same time as a single sample; the results reported returned satisfactory z-scores in both samples.

Table 11 Summary of Participants' Results and of Their Performance

Lab. code	S1-Chlorophyll a µg/L	S2-Chlorophyll a µg/L
A.V.	9.08	9.08
H.V	9.0	9.0
1	9.5	8.2
2	5.2	4.8
3	8.7	7.6
4	7.98	8.43
5	9.02	8.99
6	10.01	9.14
7	57.4	NT

Table 11 Summary of Participants' Results and of Their Performance (continued)

Lab. code	S1-Chlorophyll a µg/L	S2-Chlorophyll a µg/L
8	8.72	9.09
9	9.45	10.0
10	8	6
11	7.25	6
12	9.9	9.0
13	11	11
14	9.33	10.33
15	9.56	7.32
16	9.463	10.23
17	10.45	9.33
18	7.9	NT
19	9.76	NT
20	9.79	NT
22	9.5	9.4
23	8.9	NT
24	9.62	NT
25	9.97	9.77
26	9.7	9
27	9.53	9.59
28	9.42	9.69
29	14.91	15.24
30	10	9
31	8.2	8.1
32	9.4	8.7
33	9.5	8.0
34	9.6	8.3
35	9.47	8.59

A.V. = Assigned Value, H.V. = Homogeneity Value. Shaded cells are results which returned a questionable or unsatisfactory z-score.

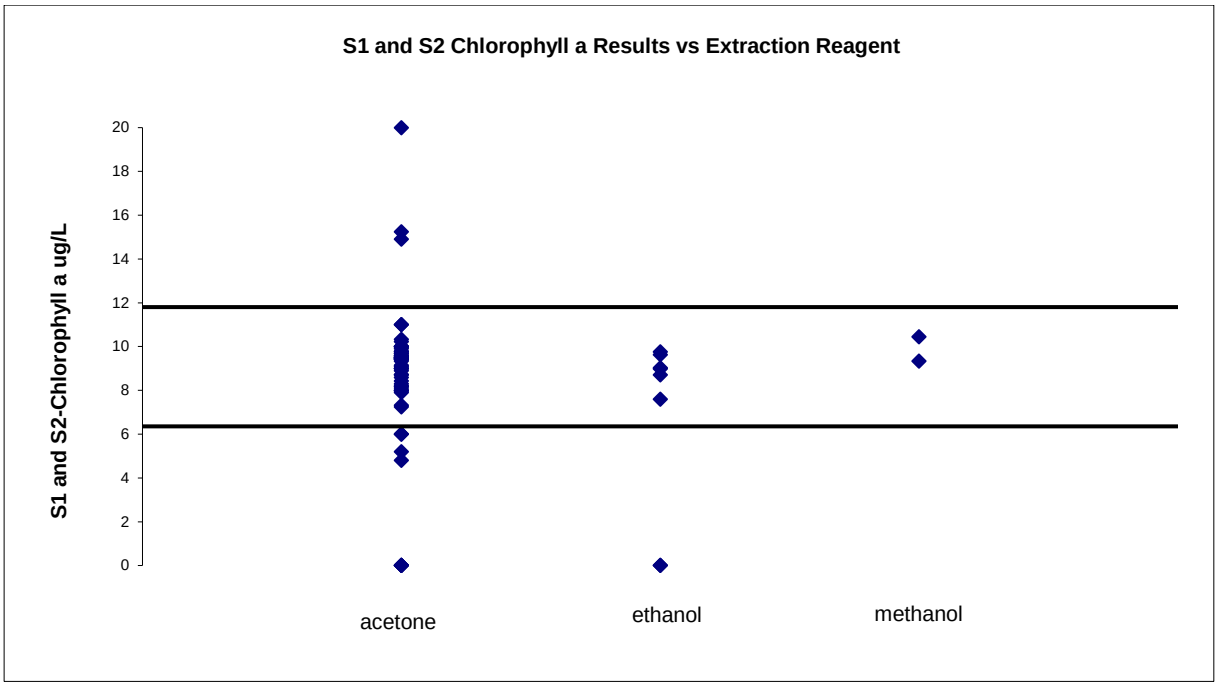
Extraction Agent

There is a wide variety of agents used for extraction of chlorophyll a and its degradation products: 90% acetone, 90% methanol, an acetone – dimethyl sulphoxide mixture, or 90% ethanol. In the present study, participants were requested to analyse the samples using their normal test method but to use the specified extraction solution: 90% (v/v) acetone.

All but five participants used 90% (v/v) acetone as instructed. Four participants used 90% or 96% ethanol and one reported using 90% methanol.

One laboratory used acetone as instructed but heating as the disruption method.

Plots of participants' results versus extraction agent are presented in Figure 8. There was no significant difference between chlorophyll a results from acetone extraction versus ethanol or methanol extraction.

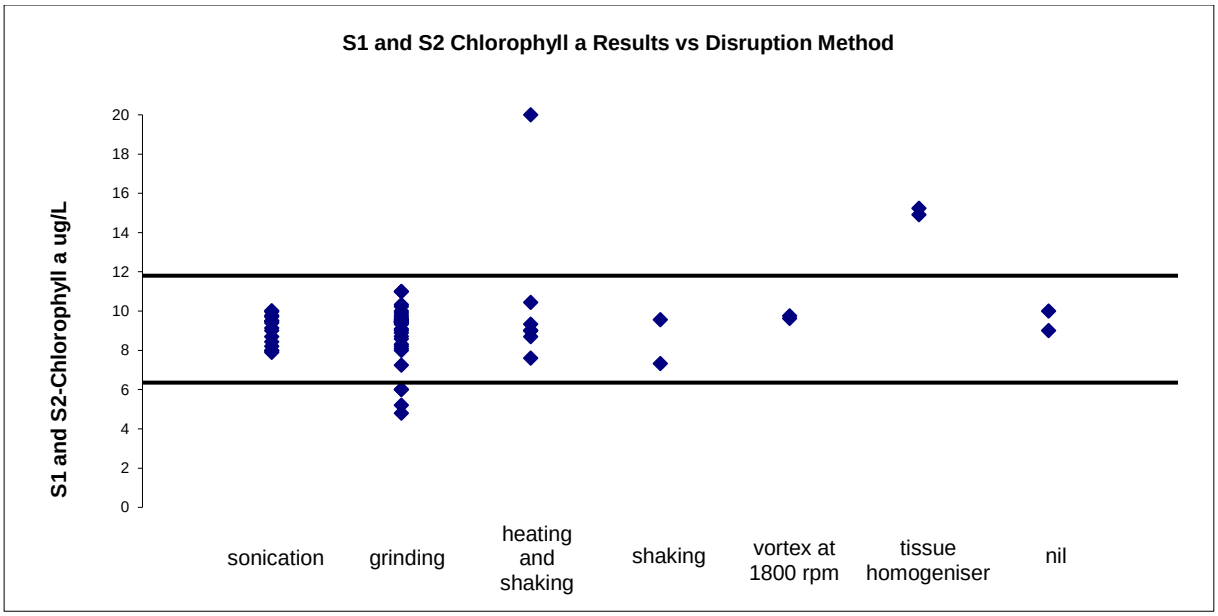


Horizontal lines on charts are the results corresponding to z-scores of 2 and -2. Laboratory 7 result was plotted as 20 µg/L.

Figure 8 Results vs. Extraction Reagent

Disruption methods

Extraction was generally aided by either grinding or sonication; one laboratory did not use a disruption method for chlorophyll a extraction. Figure 9 presents plots of participants' results versus disruption method.



Horizontal lines on charts are the results corresponding to z-scores of 2 and -2. Laboratory 7 result was plotted as 20 µg/L.

Figure 9 Results vs. Disruption Method

All low unsatisfactory results were those of participants who reported grinding as disruption method. Caution should be exercised during the disruption process; although improved extraction has been reported with sonication and mechanical grinding, both disruption procedures have also been found to increase the risk of chlorophyll a degradation. [15]

Plots of participants' results versus grinding time are presented in Figure 10.

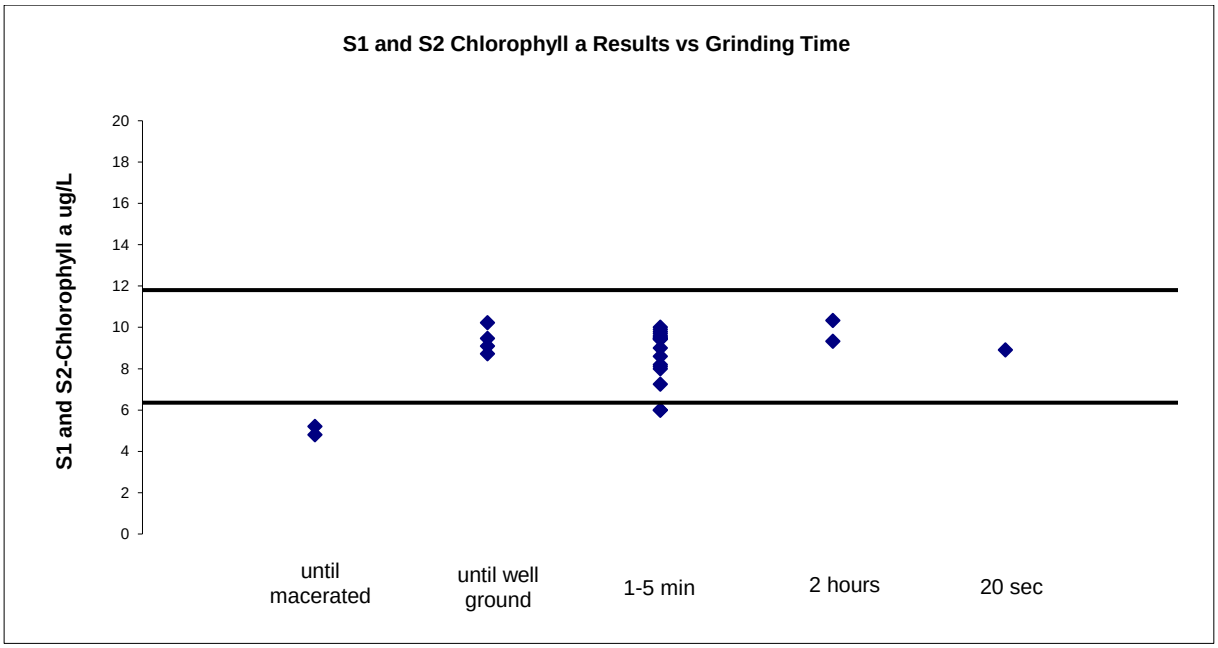
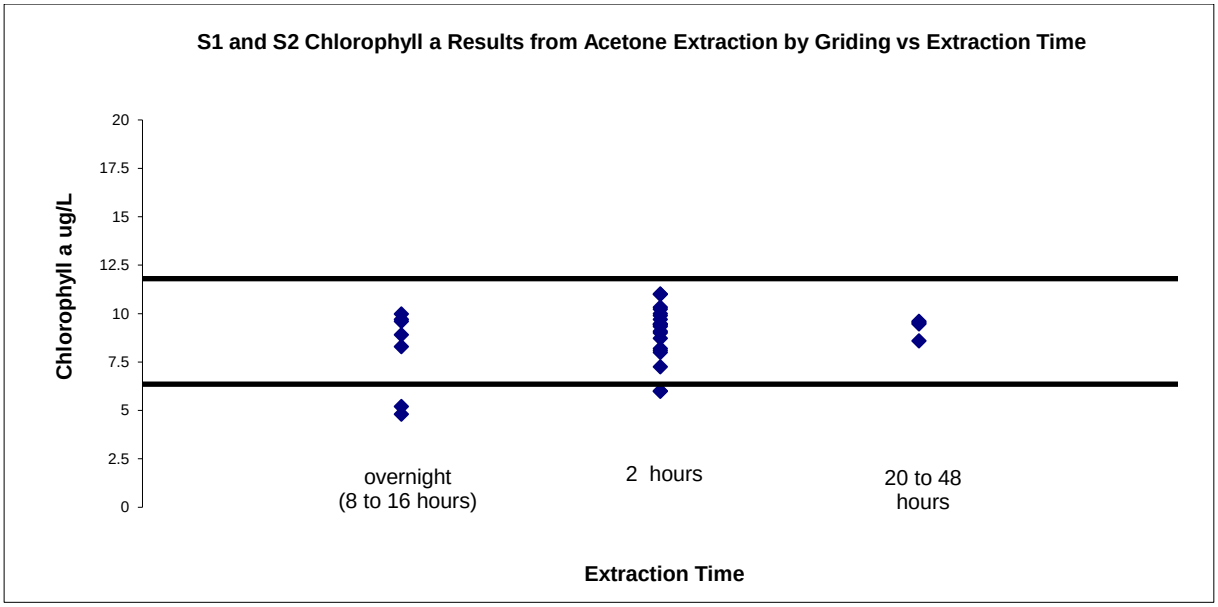


Figure 10 Results vs. Grinding Time

Extraction Time

Participants reported using various extraction times ranging from 2 to 48 hours. Plots of participants' results from the same extraction reagent and disruption method versus extraction time are presented in Figures 11 to 13.

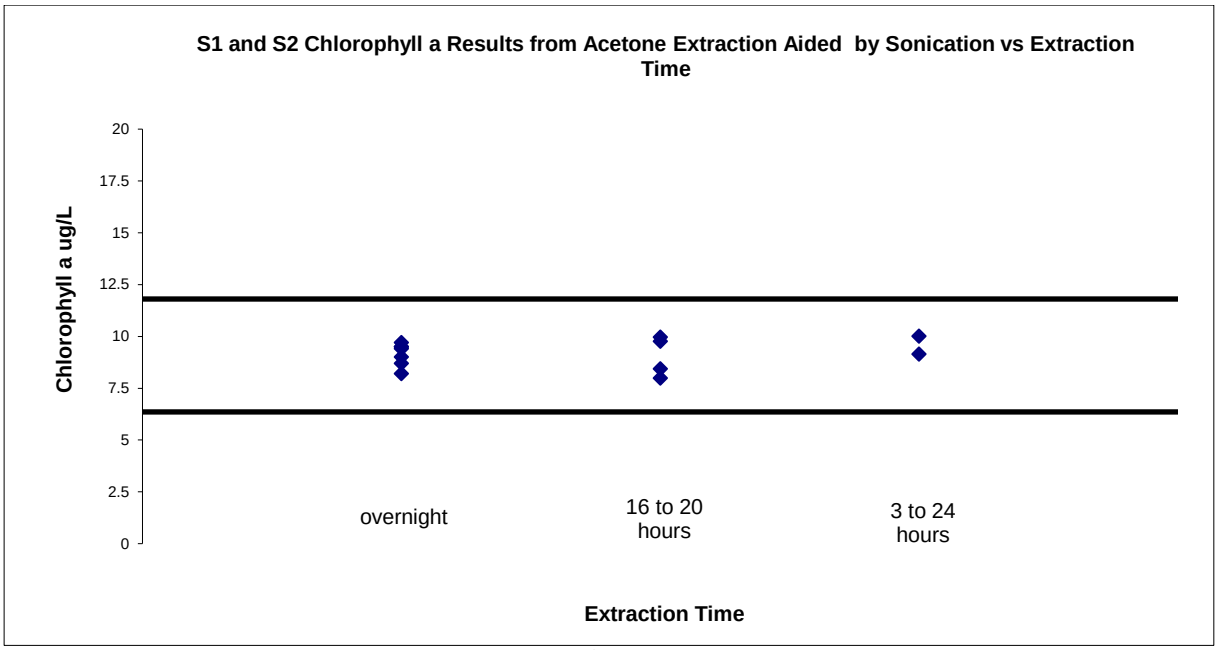
The majority of laboratories that reported using griding as disruption method extracted their samples for 2 hours (Figure 11).



Horizontal lines on charts are the results corresponding to z-scores of 2 and -2.

Figure 11 Chlorophyll a Results from Acetone Extraction Aided by Grinding vs. Extraction Time

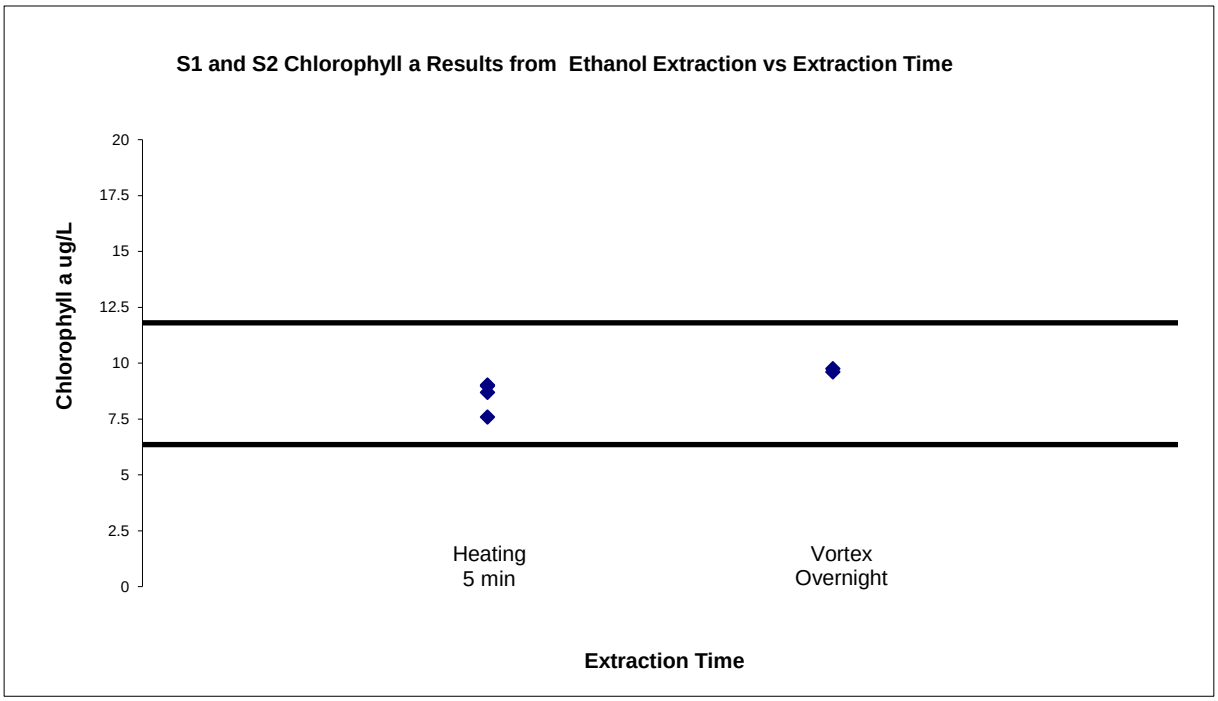
Participants who used sonication as disruption method, further extracted their samples overnight or for more than 16 hours (Figure 12).



Horizontal lines on charts are the results corresponding to z-scores of 2 and -2.

Figure 12 Chlorophyll a Results from Acetone Extraction Aided by Sonication vs. Extraction Time

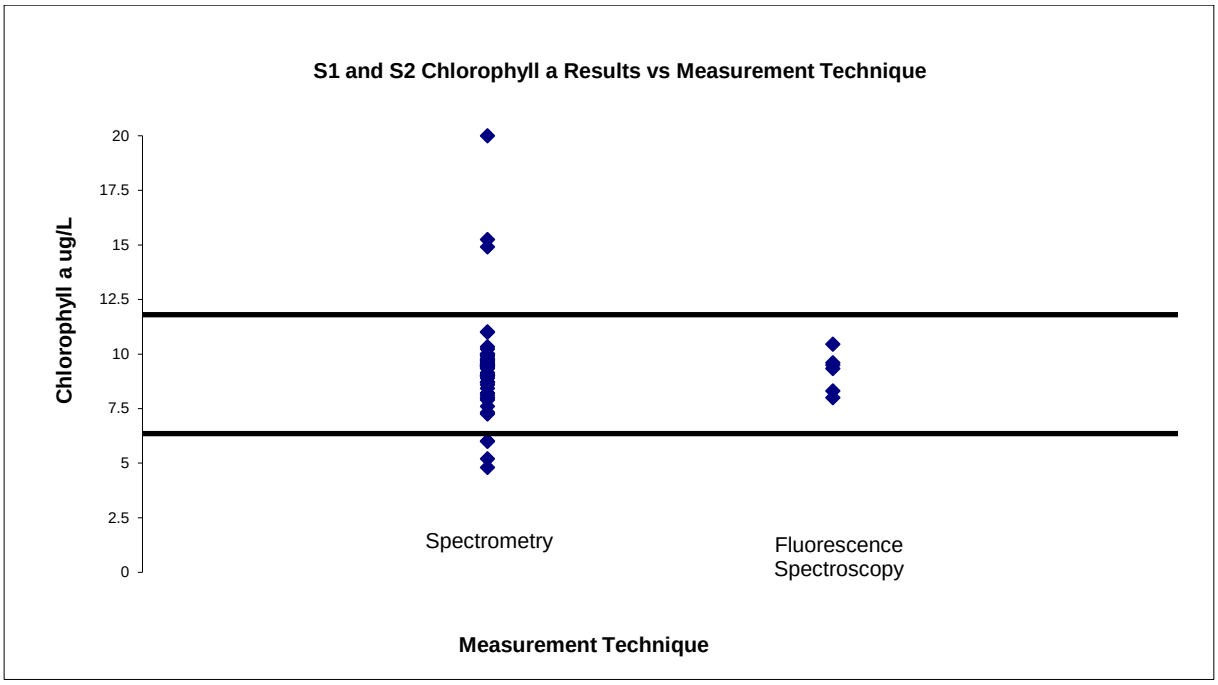
Four participants reported using ethanol for extraction, two of them shook the sample gently and heated it for 5 minutes and two vortexed it for 1 minute and then left it overnight for extraction (Figure 13).



Horizontal lines on charts are the results corresponding to z-scores of 2 and -2.

Figure 13 Chlorophyll a Results from Ethanol Extraction vs. Extraction Time

Measurement Technique
Thirty-one laboratories reported using a spectrophotometric method and 3 used fluorescence spectroscopy. A plot of chlorophyll a results versus measurement technique is presented in Figure 14.



Horizontal lines on charts are the results corresponding to z-scores of 2 and -2.

Figure 14 Chlorophyll a Results vs. Measurement Technique

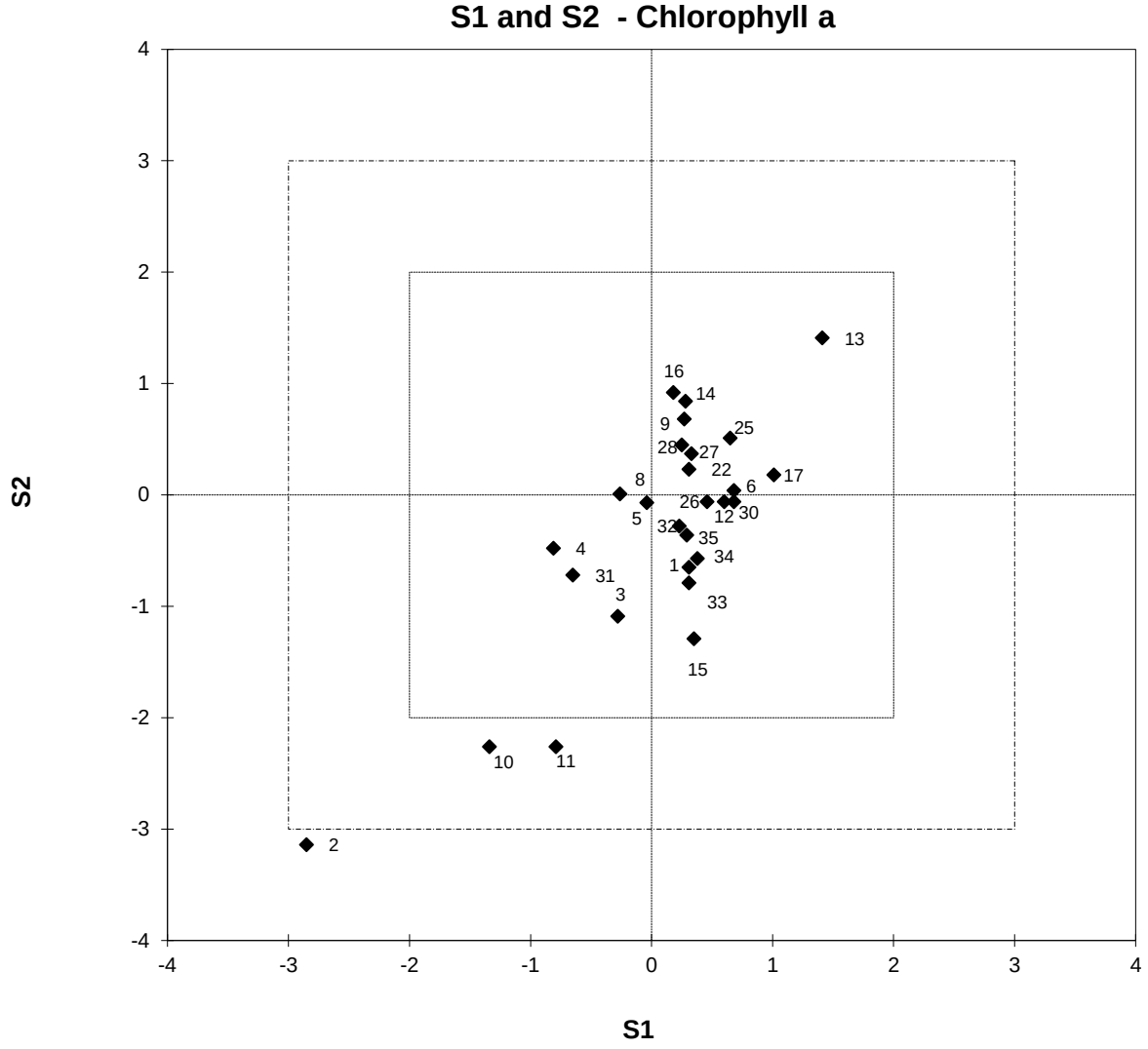
6.6 Participants' Within – Laboratory Repeatability

Samples S1 and S2 were blind duplicate samples. The same target standard deviation was used to calculate z-scores for Chlorophyll a in both samples. This allowed evaluation of participants' within laboratory repeatability.

Scatter plots of z-scores for S1 and S2 are presented in Figure 14. Points close to the diagonal axis represent excellent repeatability and points close to zero represent excellent accuracy and repeatability.

Laboratories 2, 10, 11 and 29 should check for method or laboratory bias.

Chlorophyll a measurement is challenging, as it is sensitive to light and oxygen, and to avoid oxidative and photochemical destruction the samples should not be exposed to bright light or air during analysis.[15] Most laboratories are plotted in the inner quadrant indicating that they have successfully overcome these problems.



Laboratory 29 is off scale

Figure 15 z-Score Scatter Plots for Chlorophyll a in S1 and S2

6.7 Comparison with Previous NMI Proficiency Studies of Chlorophyll a in Water

AQA 19-05 is the third NMI proficiency test of Chlorophyll a in water. Despite a lower concentration of chlorophyll a in the test samples, on average participants' performance has improved over time. The concentration of chlorophyll a in the first study conducted for chlorophyll a in water (AQA 15-22) was 25.5 µg/L and between laboratory CV was large, 17% ; in the present study the chlorophyll a level was much lower (9.08 µg/L) and the between laboratory CV was 9.8%.

Individual performance history reports are emailed to each participant at the end of the study; the consideration of z-scores for an analyte over time provides much more useful information than a single z-score.

Over time laboratories should expect at least 95% of their scores to lie within the range $|z| \leq 2$. Scores in the range $2 < |z| < 3$ can occasionally occur, however these should be interpreted in conjunction with other scores obtained by that laboratory. For example, a trend of z-scores on one side of the zero line is an indication of method or laboratory bias.

6.8 Reference Materials and Certified Reference Materials

Participants reported whether control samples (spiked samples, certified reference materials-CRMs or matrix specific reference materials-RMs) had been used (Table 12).

Table 12 RMs and CRMs Used by Participants

Lab Code	Description of Control Sample
6	Sigma Aldrich chlorophyll from spinach
8	Sigma 1 mg chlorophyll standard
10	Sigma chlorophyll standard
12	Sigma Aldrich C5753 – Chlorophyll a from spinach
14	Reference Chlorophyll a
16	CRM – LCS
17	Ultra-pure water
19	RO water blank
23	In house reference
24	Ultra-pure water
25	Reference – LRM
27	Reference material
29	Blank
31	Sigma 1 mg chlorophyll standard
35	Reference material

7 REFERENCES

[1] ISO17043:2010, Conformity assessment – *General requirements for proficiency testing*.

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[3] NMI 2016, *NMI Chemical Proficiency Testing Statistical Manual*, viewed 22 March 2017, <http://www.measurement.gov.au>.

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[5] ISO 13528:2015(E), *Statistical methods for use in proficiency testing by interlaboratory comparisons*.

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[9] Betil, M, Naykki, T, Hovind, H & Krysell, M 2004, *Nordtest Report Handbook for Calculation of Measurement Uncertainty in Environmental Laboratories*, Nordest Tekniikantie, Finland, Esopo.

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[12] ISO (2008), *Guide to the Expression of Uncertainty in Measurement (GUM)*, Geneva, Switzerland.

[13] Eurolab 2002, Technical Report No 1/2002 - *Measurement Uncertainty in Testing.*

[14] NMI, *Estimating Measurement Uncertainty for Chemists* – viewed 22 March 2017, <www.measurement.gov.au>.

[15] Holm-Hansen, O & Riemann, B 1978, "Chlorophyll a determination: improvements in methodology", *Oikos,* vol 30, pp 438-447.

APPENDIX 1 - SAMPLE PREPARATION, ANALYSIS AND HOMOGENEITY TESTING

A1.1 Sample Preparation

Samples S1 and S2, each consisted of three glass fibre filters. A chlorophyll a standard was diluted to an appropriate concentration in 90% (v/v) acetone solution. This standard solution was then used to spike each filter. All preparation was conducted under subdued light. The two study samples were blind duplicates; chlorophyll a concentration in the two samples was identical.

A1.2 Sample Analysis and Homogeneity Testing

Homogeneity testing was conducted for chlorophyll a. Seven samples (each consisting of three filters analysed separately) were analysed by a subcontracted laboratory and the average of the results was reported as the homogeneity value for chlorophyll a.

Since the entire sample was used in each analysis, it was not possible to apply analysis of variance (ANOVA) to determine if samples were sufficiently homogeneous. When it is not possible to conduct replicate measurements, the standard deviation of the results (sd) will be compared with the target standard deviation of the PT (σ) calculated as described in section 4.5. The proficiency test samples may be considered sufficiently homogeneous if: $sd \leq 0.3\ \sigma$.[5]

Data from the homogeneity testing is presented in the table below. The between sample sd as CV was 4.4 % less than 30% - the target standard deviation as CV set for this study (15%). [5]

The samples were found to be sufficiently homogeneous for use.

Table 13 Chlorophyll a Homogeneity Data

Sample number	Filter 1 Result (ug/L)	Filter 2 Result (ug/L)	Filter 3 Result (ug/L)	Average Result
S2-13	8.4	8.9	8.3	8.53
S2-37	8.2	9.3	8.1	8.53
S2-50	9	9.6	8.7	9.10
S1-42	9.1	9	9.4	9.17
S1-07	9.6	7.7	8.8	8.70
S2-29	9.8	9.5	9.1	9.47
S1-16	9.3	9.4	9.5	9.40
			Overall Average	**8.99**
			CV	**4.4%**

	Value	Critical (<30% of Target CV)	Result
CV	4.4%	4.5%	**Pass**

Sample Analysis for Chlorophyll a

Measurements for chlorophyll a for homogeneity testing were performed by ChemCentre; ChemCentre holds third party (NATA) accreditation to ISO 17025 for this test. Briefly the method used involves grinding the sample in 90% (v/v) acetone followed by extracting at 4ºC for 2 hours. The resulting solution is filtered and analysed using UV-Vis at the varying wavelengths. All measurements were carried out using a 2 cm cuvette.

APPENDIX 2 - ASSIGNED VALUE, Z-SCORE AND EN SCORE CALCULATION

Assigned value

The assigned value was calculated as the robust average using the procedure described in 'ISO13258:2015(E), Statistical methods for use in proficiency testing by interlaboratory comparisons – Annex C[5] the uncertainty was estimated as:

$$u_{rob\ av} = 1.25 * S_{rob\ av} / \sqrt{p} \qquad \text{Equation 3}$$

where:

$u_{rob\ av}$ robust average standard uncertainty
$S_{rob\ mean}$ robust average standard deviation
p number of results

The expanded uncertainty ($U_{rob\ av}$) is the standard uncertainty multiplied by a coverage factor of 2 at approximately 95% confidence level.

A worked example is set out below in Table 14.

Table 14 Uncertainty of Assigned Value for Chlorophyll a in Samples S1 and S2.

No. results (p)*	59
Assigned Value*	9.08 ug/L
$S_{rob\ av}$*	0.92 ug/L
$u_{rob\ av}$	0.16 ug/L
k	2
$U_{rob\ av}$	0.31 ug/L

[a]The assigned value was calculated as the robust average of the combined results of duplicate Samples S1 and S2 excluding Laboratories, 7 and 29.

The assigned value for **Chlorophyll a** in Samples S1 and S2 is **9.08 ± 0.31 ug/L**.

z-Score and E_n-score

For each participant's result a z-score and E_n-score are calculated according to Equation 1 and Equation 2 respectively (see page 11).

A worked example is set out below in Table 15.

Table 15 z-Score and E_n-score for Chlorophyll a Result Reported by Laboratory 1 in S1

Chlorophyll a Result ug/L	Assigned Value ug/L	Set Target Standard Deviation	z-Score	E_n-Score
9.5 ± 1.9	9.08 ± 0.31	15% as CV or 0.15 x 9.08 = 1.36 ug/L	$z = \frac{(9.5 - 9.08)}{1.36}$ z = 0.31	$En = \frac{(9.5 - 9.08)}{\sqrt{1.9^2 + 0.31^2}}$ E_n = 0.22

APPENDIX 3 - STABILITY STUDY

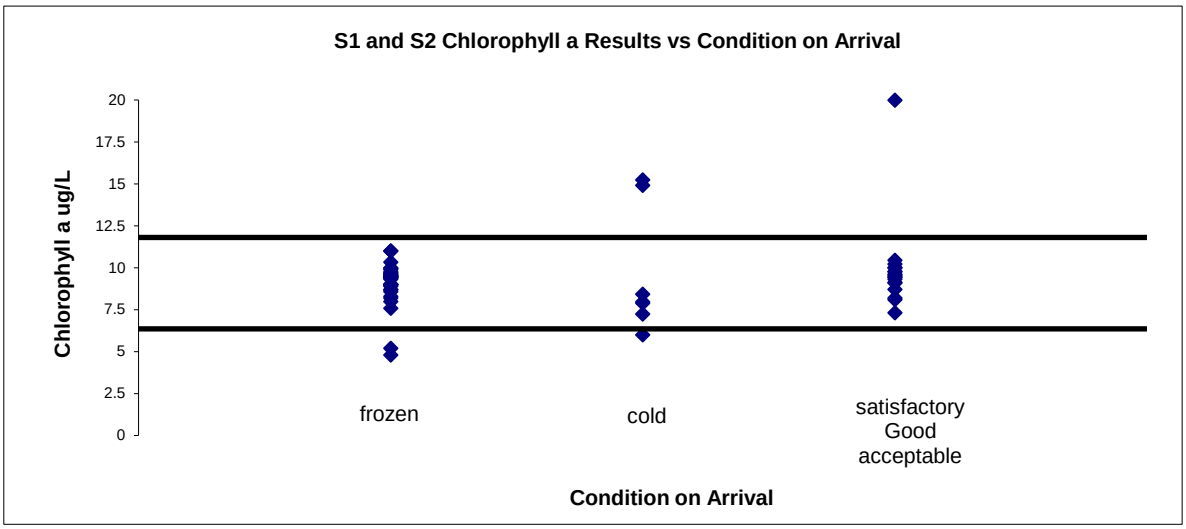
Participants were advised to store the samples frozen if analysis cannot be commenced on the day of receipt, subdued light conditions were also advised for all procedures. The samples condition on receipt and the date when the sample was received and analysed by the participants are presented in Table 16

Table 16 Sample Condition on Receipt and the Date when the Sample was received and Analysed

Lab Code	Received Date	Arrival Condition	Analysis Date
1	27/03/2019	frozen	29/03/2019
2	26/03/2019	frozen	01/04/2019
3	29/03/2019	frozen	29/03/2019
4	26/03/2019	cold	27/03/2019
5	26/03/2019	Frozen	28/03/2019
6	26/03/2019	good	27/03/2019
7	27/03/2019	Good	28/03/2019
8	26/03/2019	Satisfactory	27/03/2019
9	26/03/2019	Acceptable	28/03/2019
10	26/03/2019	Good	04/04/2019
11	26/03/2019	Cold	27/03/2019
12	26/03/2019	Frozen	03/04/2019
13	26/03/2019	Frozen	08/04/2019
14	26/03/2019	frozen	26/03/2019
15	25/03/2019	good	29/03/2019
16	26/03/2019	OK	01/04/2019
17	28/03/2019	Satisfactory	04/04/2019
18	27/03/2019	Cold	27/03/2019
19	26/03/2019	Good	27/03/2019
20*	09/04/2019	Good	10/04/2019
22	27/03/2019	Frozen	04/04/2019
23	26/03/2019	Frozen	27/03/2019
24	27/03/2019	Good	04/04/2019
25	26/03/2019	Frozen	26/03/2019
26	26/03/2019	Frozen	01/04/2019
27	26/03/2019	Frozen	01/04/2019
28	26/03/2019	Frozen	04/04/2019
29	26/03/2019	Cool	27/03/2019
30	26/03/2019	Frozen	27/03/2019
31**	02/04/2019	Good	02/04/2019
32	27/03/2019	Frozen	29/03/2019
33	27/03/2019	Frozen	29/03/2019
34	27/03/2019	Frozen	29/03/2019
35	26/03/2019	Frozen	01/04/2019
Homogeneity Testing (T0)	26/03/2019	Frozen	02/04/2019
Stability Testing (T16)*	09/04/2019	Frozen	10/04/2019

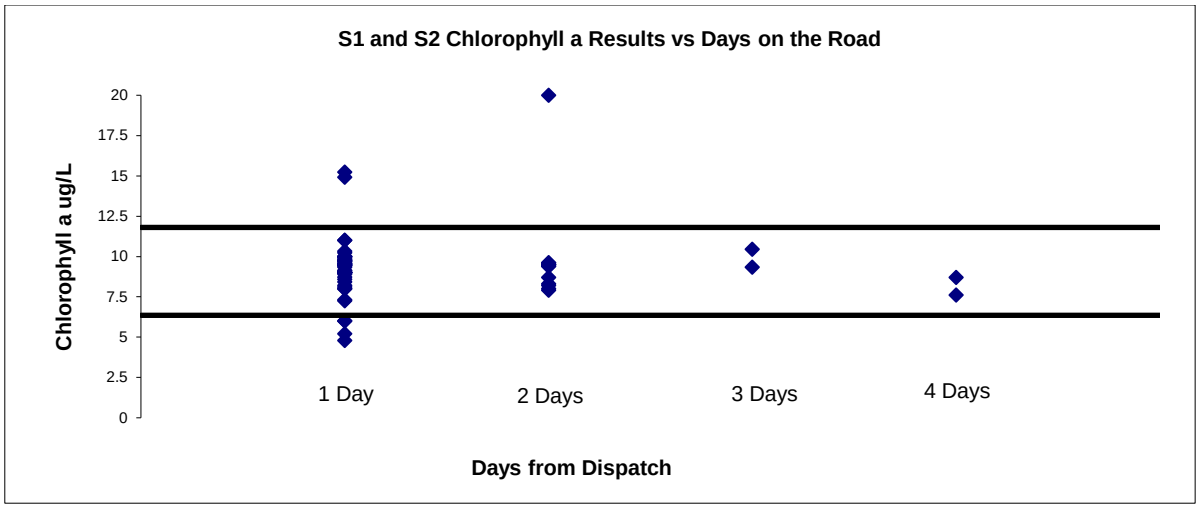
*The samples have been dispatched on 08.04.2019; *The samples have been dispatched on 01.04.2019

No correlation between reported chlorophyll a result, the number of days on the road, the analysis date or the sample condition on arrival was observed (Figures 15 to 17).



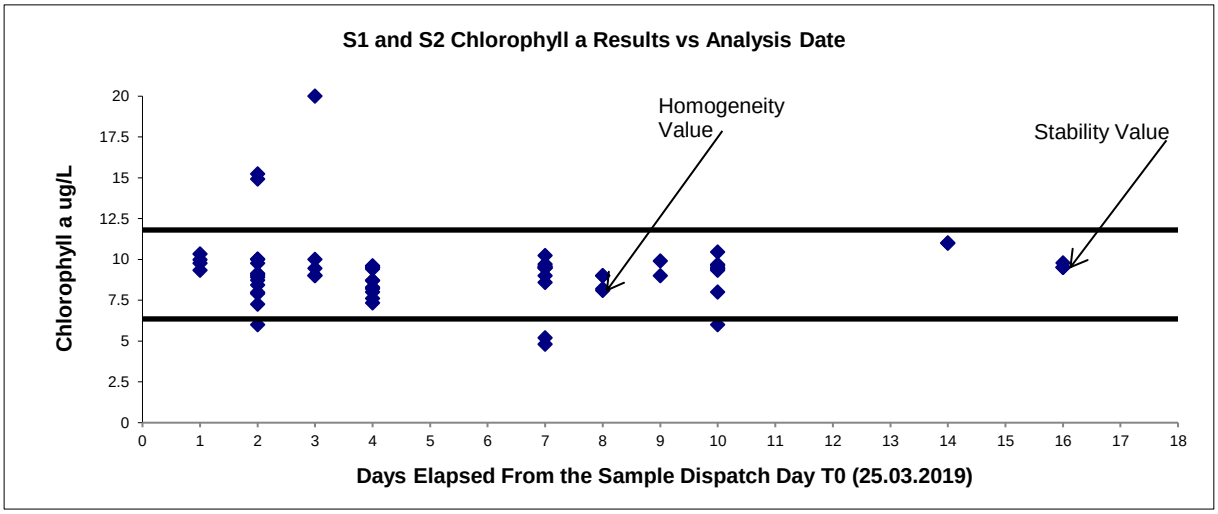
Horizontal lines on charts are the results corresponding to z-scores of 2 and -2. Laboratory 7 result has been plotted as 20 ug/L.

Figure 16 Chlorophyll a Concentration vs. Condition on Arrival



Horizontal lines on charts are the results corresponding to z-scores of 2 and -2. Laboratory 7 result has been plotted as 20 ug/L.

Figure 17 Chlorophyll a Concentration vs. Days on the Road



Horizontal lines on charts are the results corresponding to z-scores of 2 and -2. Laboratory 7 result has been plotted as 20 ug/L.

Figure 18 Chlorophyll a Concentration vs. Analysis Date

Stability Study

Stability studies conducted for chlorophyll a in water in previous studies found no significant changes in concentration. [6, 7] However, a stability study was conducted in the present study. The analyses were carried out over the entire period of study: when the study started (T0) and at its end, 16 days later (T16).

A Student t-test was used to compare the two sets of results. No significant change in chlorophyll a concentration in the elapsed time was evident (p=0.068).

The chlorophyll a results at (T0) and (T16) were also in good agreement with the assigned value and spike value within their stated uncertainties (Figure 18).

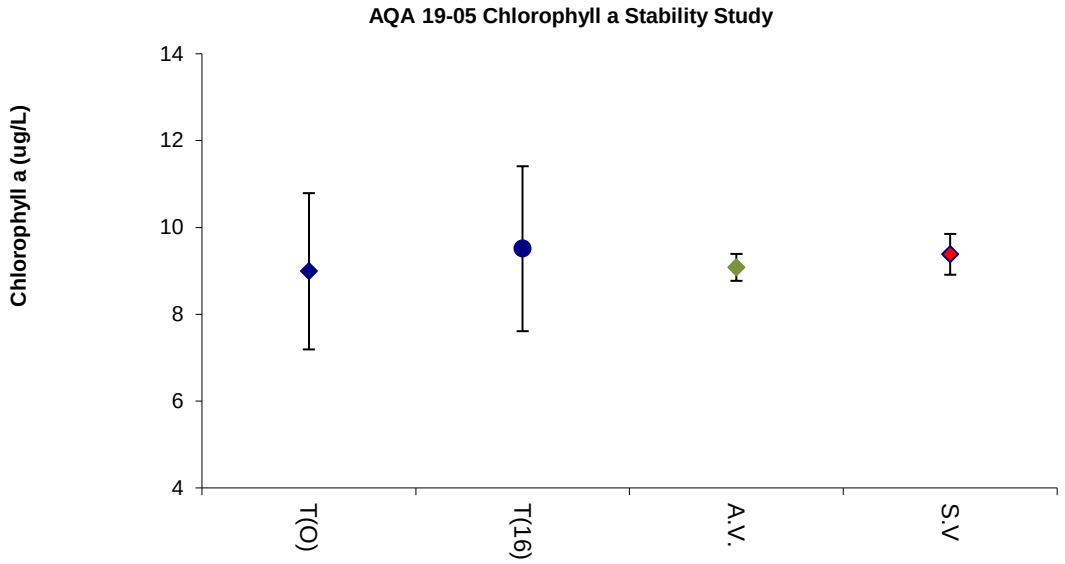


Figure 19 Chlorophyll a Stability Results

APPENDIX 4 - ACRONYMS AND ABBREVIATIONS

HV	Homogeneity Value
Max	Maximum value in a set of results
Md	Median
Min	Minimum value in a set of results
NMI	National Measurement Institute (of Australia)
NR	Not Reported
NT	Not Tested
PT	Proficiency Test
PCV	Performance Coefficient of Variation
RA	Robust Average
RM	Reference Material
Robust CV	Robust Coefficient of Variation
Robust SD	Robust Standard Deviation
S	Spiked or formulated concentration of a PT sample
SI	The International System of Units
s^2_{sam}	Sampling variance
s_a/σ	Analytical standard deviation divided by the target standard deviation
SRM	Standard Reference Material (Trademark of NIST)
Target SD	Target standard deviation
σ	Target standard deviation

APPENDIX 5 – PARTICIPANTS RESULTS

Table 17 Chlorophyll a Results in S1

Lab Code	Filter 1 (ug/L)		Filter 2 (ug/L)		Filter 3 (ug/L)		Average (ug/L)	
	Results	Uncertainty	Results	Uncertainty	Results	Uncertainty	Results	Uncertainty
1	8.9	1.8	10.1	2	9.6	1.9	9.5	1.9
2	5.2	0.5	4.8	0.5	5.6	0.5	5.2	0.5
3	8.5	0.8	8.6	0.8	9	0.8	8.7	0.8
4	8.29	1.62	8.39	1.64	7.26	1.42	7.98	1.56
5	9.08	1.56	9.09	1.56	8.88	1.53	9.02	1.55
6	10.1	0.86	9.99	0.85	9.94	0.84	10.01	0.85
7	80.5	NR	44	NR	47.6	NR	57.4	NR
8	9.83	1.39	8.33	1.18	8.01	1.13	8.72	1.23
9	9.37	1.41	9.61	1.44	9.37	1.41	9.45	1.42
10	8	4	8	4	8	4	8	4
11	5	4	7	4	9	4	7.25	4
12	10.3	4.1	9.7	3.9	9.7	3.9	9.9	3.9
13	11	NR	11	NR	11	NR	11	NR
14	NR	NR	NR	NR	NR	NR	9.33	2.748
15	9.4	1.4	9.77	1.5	9.67	1.4	9.56	1.4
16	9.17	1.26	10.01	1.36	9.21	1.26	9.463	1.29
17	10.31	NR	10.64	NR	10.38	NR	10.45	0.53
18	8.1	6.67	7.8	6.67	7.8	6.67	7.9	6.67
19	9.94	1.7	9.63	1.65	9.72	1.66	9.76	1.67
20	10.146	1	9.879	1	9.345	0.9	9.79	1
22	9.5	14.2	9.7	14.2	9.2	14.2	9.5	14.2
23	8.8	0.9	8.8	0.9	9	0.9	8.9	0.9
24	9.86	1.69	9.29	1.59	9.71	1.66	9.62	1.65
25	10	0:00	10	1	9.9	0.99	9.97	1.00
26	10	NR	9	NR	10	NR	9.7	NR
27	9.65	0.264	9.38	0.264	9.56	0.264	9.53	0.264
28	9.75	2.44	9.4	2.35	9.1	2.28	9.42	2.36
29	12.02	4	17.69	4	15.02	4	14.91	4
30	10	1.5	10	1.5	10	1.5	10	1.50
31	7.4	1.01	7.2	23:31	10.1	8:52	8.2	1.12
32	9.1	1.8	9.5	1.9	9.7	1.9	9.4	1.9
33	9.2	2.8	9.6	2.9	9.6	2.9	9.5	2.8
34	9.2	2.8	9.8	2.9	9.8	2.9	9.6	2.9
35	9.75	0.264	9.55	0.264	9.11	0.264	9.47	0.264

Table 18 Pheophytin a Results in S1

Lab Code	Filter 1 (ug/L)		Filter 2 (ug/L)		Filter 3 (ug/L)		Average (ug/L)	
	Results	Uncertainty	Results	Uncertainty	Results	Uncertainty	Results	Uncertainty
1	<4	NR	<4	NR	<4	NR	<4	NR
2	12.2	1	12.6	1	13.2	1	12.7	1
3	1.6	0.3	1.9	0.3	1.5	0.2	1.7	0.3
4	0.98	0.5	0.83	0.41	1.56	0.8	1.12	0.56
5	NT	NT	NT	NT	NT	NT	NT	NT
6	<0.2	NA	<0.2	NA	<0.2	NA	<0.2	NR
7	NR	NR	NR	NR	NR	NR	NR	NR
8	<1	0.14	<1	0.14	1.04	0.15	<1	0.14
9	0.39	0.06	0.82	0.12	0.72	0.11	0.64	0.10
10	2	2	2	2	2	2	2	2
11	2	2	3	2	2	2	2.5	2
12	<1	NR	<1	NR	<1	NR	<1	NR
13	NR	NR	NR	NR	NR	NR	NR	NR
14	NR	NR	NR	NR	NR	NR	<1	NR
15	NT	NT	NT	NT	NT	NT	NT	NT
16	<1	0.5	<1	0.5	<1	0.5	<1	0.5
17	NR	NR	NR	NR	NR	NR	NT	NT
18	NT	NT	NT	NT	NT	NT	NT	NT
19	9.62	3.29	9.18	3.14	9.18	3.14	9.32	3.19
20	<5	1	<5	1	<5	1	<5	1
22	0	NR	0	NR	0	NR	0.0	NR
23	1	0.5	0.9	0.5	0.9	0.5	0.9	0.5
24	0.96	0.33	1.73	0.59	1.29	0.44	1.33	0.45
25	NT	NT	NT	NT	NT	NT	NT	NT
26	NR	NR	NR	NR	NR	NR	NR	NR
27	<2.0	0.255	<2.0	0.255	<2.0	0.255	<2.0	0.255
28	<0.5	0.5	<0.5	0.5	<0.5	0.5	<0.5	0.5
29	<0.5	4	<0.5	4	<0.5	4	<0.5	4
30	<2	NR	<2	NR	<2	NR	<2	NR
31	NT	NT	NT	NT	NT	NT	NT	NT
32	NT	NT	NT	NT	NT	NT	NT	NT
33	<4	NR	<4	NR	<4	NR	<4	NR
34	NT	NT	NT	NT	NT	NT	NT	NT
35	<2.0	0.255	<2.0	0.255	<2.0	0.255	<2.0	0.255

Table 19 Chlorophyll a Results in S2

Lab Code	Filter 1 (ug/L)		Filter 2 (ug/L)		Filter 3 (ug/L)		Average (ug/L)	
	Results	Uncertainty	Results	Uncertainty	Results	Uncertainty	Results	Uncertainty
1	7.3	1.5	8.3	1.7	9	1.8	8.2	1.6
2	4.8	0.5	3.6	0.5	6	0.5	4.8	0.5
3	6.6	0.6	8.7	0.8	7.6	0.7	7.6	0.7
4	8.39	1.64	8.43	1.65	8.48	1.66	8.43	1.65
5	9.13	1.57	9.11	1.57	8.72	1.5	8.99	1.55
6	9.45	0.8	9	0.76	8.97	0.76	9.14	0.77
7	NT	NT	NT	NT	NT	NT	NT	NT
8	8.97	1.27	9.64	1.36	8.65	1.22	9.09	1.28
9	10.3	1.55	10.1	1.52	9.61	1.44	10.0	1.5
10	6	3	6	3	6	3	6	3
11	7	3	5	3	6	3	6	3
12	9.3	3.7	9	3.6	8.8	3.5	9.0	3.6
13	11	NR	11	NR	11	NR	11	NR
14	NR	NR	NR	NR	NR	NR	10.33	3.033
15	5.61	1.4	8.54	2.1	7.8	2	7.32	1.8
16	10.39	1.4	9.91	1.35	10.39	1.4	10.23	1.38
17	9.12	NR	10.38	NR	8.48	NR	9.33	2.91
18	NT	NT	NT	NT	NT	NT	NT	NT
19	NT	NT	NT	NT	NT	NT	NT	NT
20	NT	NT	NT	NT	NT	NT	NT	NT
22	9.8	14.2	9.7	14.2	8.8	14.2	9.4	14.2
23	NT	NT	NT	NT	NT	NT	NT	NT
24	NT	NT	NT	NT	NT	NT	NT	NT
25	9.9	0.99	9.8	0.98	9.6	0.96	9.77	0.98
26	9	NR	9	NR	9	NR	9	NR
27	9.31	0.264	9.74	0.264	9.72	0.264	9.59	0.264
28	9.8	2.45	9.48	2.37	9.8	2.45	9.69	2.42
29	16.02	4	15.35	4	14.35	4	15.24	4
30	9	1.35	8	1.2	9	1.35	9	1.35
31	7.5	1.02	7.8	1.06	9	1.22	8.1	1.01
32	7.8	1.6	8.9	1.8	9.2	1.8	8.7	1.7
33	6.5	2	8.6	2.6	8.8	2.6	8.0	2.4
34	6.7	2	8.8	2.6	9.2	2.8	8.3	2.5
35	8.73	0.264	9.66	0.264	7.39	0.264	8.59	0.264

Table 20 Pheophytin a Results in S2

Lab Code	Filter 1 (ug/L)		Filter 2 (ug/L)		Filter 3 (ug/L)		Average (ug/L)	
	Results	Uncertainty	Results	Uncertainty	Results	Uncertainty	Results	Uncertainty
1	<4	NR	<4	NR	<4	NR	<4	NR
2	14	1.5	15.7	1.5	11.7	1	13.8	1
3	2.7	0.4	1.7	0.3	2.5	0.4	2.3	0.4
4	0.68	0.34	0.78	0.39	0.9	0.45	0.79	0.4
5	NT	NT	NT	NT	NR	NR	NT	NT
6	<0.2	NA	<0.2	NA	0.48	0.08	<0.2	NR
7	NT	NT	NT	NT	NT	NT	NT	NT
8	<1	0.14	<1	0.14	<1	0.14	<1	0.14
9	0	NR	0	NR	0.48	0.07	0.16	0.02
10	2	2	3	3	3	3	3	3
11	1	2	4	2	3	2	3	2
12	<1	NR	<1	NR	<1	NR	<1	NR
13	NR	NR	NR	NR	NR	NR	NR	NR
14	NR	NR	NR	NR	NR	NR	<1	NR
15	NT	NT	NT	NT	NT	NT	NT	NT
16	<1	0.5	<1	0.5	<1	0.5	<1	0.5
17	NR	NR	NR	NR	NR	NR	NT	NT
18	NT	NT	NT	NT	NT	NT	NT	NT
19	NT	NT	NT	NT	NT	NT	NT	NT
20	NT	NT	NT	NT	NT	NT	NT	NT
22	0	NR	0	NR	0.1	NR	0.0	NR
23	NT	NT	NT	NT	NT	NT	NT	NT
24	NT	NT	NT	NT	NT	NT	NT	NT
25	NT	NT	NT	NT	NT	NT	NT	NT
26	NR	NR	NR	NR	NR	NR	NR	NR
27	<2.0	0.255	<2.0	0.255	<2.0	0.255	<2.0	0.255
28	<0.5	0.5	<0.5	0.5	<0.5	0.5	<0.5	0.5
29	<0.5	4	<0.5	4	<0.5	4	<0.5	4
30	<2	NR	2	0.3	2	0.3	2	0.30
31	NT	NT	NT	NT	NT	NT	NT	NT
32	NT	NT	NT	NT	NT	NT	NT	NT
33	<4	NR	<4	NR	<4	NR	<4	NR
34	NT	NT	NT	NT	NT	NT	NT	NT

APPENDIX 6 – MEASUREMENT TECHNIQUE

Table 21 Measurement Technique for Chlorophyll a and Pheophytin a

Lab. Code	Measurement Technique
1	spectrophotometric
2	spectrophotometric
3	spectrophotometric
4	spectrophotometric
5	spectrophotometric
6	spectrophotometric
7	spectrophotometric
8	spectrophotometric
9	spectrophotometric
10	spectrophotometric
11	Spectrophotometric
12	spectrophotometric
13	spectrophotometric
14	spectrophotometric
15	spectrophotometric
16	UV/Vis
17	Fluorometric
18	spectrophotometric
19	spectrophotometric
20	spectrophotometric
22	spectrophotometric
23	spectrophotometric
24	spectrophotometric
25	spectrophotometric
26	spectrophotometric
27	spectrophotometric
28	spectrophotometric
29	spectrophotometric
30	spectrophotometric
31	spectrophotometric
32	spectrophotometric
33	Fluorometric
34	Fluorometric
35	spectrophotometric